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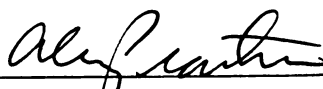
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THE REPRODUCTIVE BIOLOGY AND TAXONOMIC ORIGIN OF
MICHIGAN MONKEY-FLOWER, *MIMULUS GLABRATUS* VAR. *MICHIGANENSIS*

By

Amanda L. Posto

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ABSTRACT

THE REPRODUCTIVE BIOLOGY AND TAXONOMIC ORIGIN OF MICHIGAN MONKEY-FLOWER, *MIMULUS GLABRATUS* VAR. *MICHIGANENSIS*

By

Amanda L. Posto

Michigan monkey-flower, *Mimulus glaberratus* var. *michiganensis*, is a rare plant endemic to Michigan. Because this taxon is federally endangered and very rare there is much interest in learning more about its biology. Research on its reproductive biology focused on mating system parameters, pollen viability and seed germination. Michigan monkey-flower is self-compatible and plants from the Maple River population are capable of self-pollination. There is considerable variation (27-52%) in pollen viability between individuals of the Maple River population, which has implications for mating, selection, and gene flow within this population. The Reese's Swamp population, which had not been previously studied, has 0% viable pollen similar to other populations studied with the exception of the Maple River population. Among four seed germination regimes tested, the highest germination rates (67%) were observed at ~23°C with exposure to light. Molecular markers were used in the taxonomic analysis to test between alternative hypotheses of hybrid origin from James' monkey-flower, *M. glaberratus* var. *jamesii*, and Common monkey-flower, *M. guttatus*, versus divergence from one or the other. Michigan monkey-flower is not of recent origin and, based on genetic similarity between taxa, is most likely diverged from James' monkey-flower. Michigan monkey-flower is genetically distinct from the other taxa and has relatively high intraspecific identity compared to the more widespread James' monkey-flower and Common monkey-flower.

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CHAPTER 1

INTRODUCTION

Michigan monkey-flower, *Mimulus glabratus* var. *michiganensis* (Pennell) Fassett, is the only plant taxon endemic to Michigan. It is rare in Michigan and is known from only 15 extant localities and 3 historical sites all within the Mackinac Straits (Charlevoix, Cheboygan, Emmet and Mackinac counties) and Grand Traverse (Benzie and Leelanau counties) regions of Michigan (USFWS, 1997). It is protected under the Federal Endangered Species Act (U.S. Department of Interior, 1990) and in Michigan (Michigan Department of Natural Resources, 1991) and is most likely endangered because of shoreline development (USFWS, 1997). It is an aquatic perennial in a widespread complex of yellow monkey-flowers that are mainly distributed in the western United States. Because this taxon is federally listed and very rare there is much interest in learning more about its biology.

In this study I explore important questions about the reproductive biology and taxonomic origin of Michigan monkey-flower. My research priorities were chosen by consulting the recovery plan for this taxon (USFWS, 1997) and conferring with Michigan Natural Features Inventory staff. The investigation of the reproductive biology of Michigan monkey-flower will involve assessment of its mating system biology, pollen viability and seed germination. An understanding of the basic reproductive biology is essential for understanding the causes and consequences of rarity and will provide the foundation and focus for further studies. A study of the reproductive biology will provide information relevant to the biology of Michigan monkey-flower in the following areas:

- *Mating system biology.* Persistence of rare species depends largely on their reproductive ability (Heunneke, 1991; Barret and Kohn 1991). Furthermore, low rates of sexual reproduction are often associated with rarity, sometimes as a cause and sometimes as a consequence. Because sexual reproduction has an impact on population persistence and on the maintenance of genetic diversity, testing for self-compatibility will provide baseline data needed for management plans for this endangered species.
- *Pollen viability.* Michigan monkey-flower has very low pollen viability, less than 1%, in all but one population studied by Bliss (1983, 1986). The remaining population, the Maple River population, was reported to have 30% pollen viability. In the Maple River population pollen viability may differ considerably between individuals, but Bliss sampled only three individuals, one anther from each, per population, thus possible variation could have gone undetected. Differences in pollen viability between individuals may have important consequences for reproduction and individual fitnesses in the population.
- *Seed germination.* Fruit set is low in most populations and capsules were never full of seeds (Bliss 1986). This low level of seed production can also have important consequences for sexual reproduction. Quantifying rates of seed germination may give us insight into one of the causes of rarity of this species and may provide information critical to management planning.

Experiments to investigate the mating system of Michigan monkey-flower were conducted using individuals of the Maple River population, the only population known to have significant pollen viability and fruit set (Bliss, 1986).

The taxonomic origin of Michigan monkey-flower was investigated using molecular markers to test between the alternative hypotheses of hybrid origin from James' monkey-flower, *M. glabratus* var. *jamesii*, and Common monkey-flower, *M. guttatus*, versus divergence from one or the other. Bliss (1983, 1986) proposed three hypotheses to explain its origin: (1) that Michigan monkey-flower originated from a hybridization event between James' monkey-flower and the Common monkey-flower, (2) that Michigan monkey-flower originated from a chromosomal rearrangement of James' monkey-flower, or (3) that Michigan monkey-flower originated as a disjunct aneuploid (gain or loss of a chromosome) of the Common monkey-flower. The RAPD (Random Amplified Polymorphic DNA) technique (Welsh and McClelland 1990; Williams et al. 1990) was used to compare Michigan monkey-flower to its putative parental species, James' monkey-flower and Common monkey-flower. Understanding the genetic relationship of Michigan monkey-flower to James' monkey-flower and Common monkey-flower is important because these relationships may offer insight into the appropriate taxonomic rank for the taxon, which in turn will have implications for its level of protection.

Investigation of the reproductive biology and taxonomic origin will contribute greatly to our understanding of the biology of Michigan monkey-flower, provide baseline knowledge for prioritizing conservation programs, and lay the groundwork for future studies.

CHAPTER 2

THE REPRODUCTIVE BIOLOGY OF MICHIGAN MONKEY-FLOWER

Introduction

Michigan monkey-flower is an emergent aquatic restricted to cool, alkaline springs and streams usually associated with Northern white-cedar (*Thuja occidentalis*) swamps along current and post-glacial Great Lakes shorelines (USFWS 1997). It is a prostrate to erect herb usually found growing in muck or sandy soils and the stems often root at the lower nodes to produce numerous shoots via stolons. It typically flowers from mid-June to late August.

Bliss (1986) conducted a reproductive study of Michigan monkey-flower focusing on pollen viability and fruit set. She documented less than one percent pollen viability in seven populations and thirty percent pollen viability in the population at Maple River. Three plants were sampled per population and intrapopulational variation in pollen viability, if present, was not reported. Bliss observed 75% pollen viability in 14 populations of James' monkey-flower, which is more typical of angiosperms (Kearns and Inouye, 1993).

Michigan monkey-flower is reported to be self-incompatible (Bliss, 1983). However, Bliss used only plants from populations with less than 1% pollen viability in her crossing experiments. Mating crosses conducted using individuals from Maple River, the only population known to produce viable pollen (Bliss, 1983), may provide a more accurate view of the mating system of this group. Other varieties of *Mimulus glabratus* are known to be self-compatible and readily self-pollinating (Vickery, 1991).

Bliss observed, but did not quantify, seed set. She found 100% of shoots with developing fruit in the Maple River population and only 3.4% of shoots with developing fruit in the remaining populations; she noted that capsules were never full in any population. These results (Bliss, 1983, 1986) suggest that reproductive biology is an important factor in the rarity of Michigan monkey-flower. While the plants reproduce clonally, their rates of sexual reproduction are apparently very low.

In order to gain a greater understanding of the reproductive biology of Michigan monkey-flower, I measured pollen viability in multiple individuals of the Maple River population and several other populations, including individuals from Reese's Swamp, a vigorous population that had not been previously studied. In addition, I investigated the mating system of Maple River individuals and tested four regimes for measuring seed germination.

Materials and Methods

Pollen Stainability

Pollen stainability, as an estimate of pollen viability, was measured using the aceto-carmin jelly staining technique (Radford et al., 1974). Aceto-carmin jelly is a semi-permanent mounting medium in which pollen grains containing cytoplasm stain purple and are assumed to be viable. Pollen grains lacking cytoplasm are assumed to be inviable and do not stain. This method probably overestimates pollen viability (Peters et al., 1990; Kearns and Inouye, 1993).

Pollen was sampled from 17 individuals of five populations of Michigan monkey-flower: one individual from Burt Lake, five individuals from Carp Creek, four from Glen Lake, five from Maple River and two from Reese's Swamp. Bliss (1983, 1986) did not

fine statistical differences in pollen viability, estimated as pollen stainability, between anthers of a single flower therefore, one undehisced anther was sampled from a single flower per individual. The anther was removed from a bud of each individual and macerated in a drop of aceto-carmin jelly.

Pollen grains lacking cytoplasm tend to float to the edges of a coverslip, thus stained pollen may be over-represented in microscope fields that are not near the edge, while unstained pollen may be over-represented near the edge. Therefore, systematic sampling may lead to a bias, depending on the ratio of edge vs. non-edge fields that are counted. To insure that unbiased, accurate measures of pollen stainability were recorded, total counts of pollen stainability were made counting all pollen grains per slide for 5 slides. This provided a total measure of pollen viability, which was evaluated with three different methods of sampling: 1) randomized vertical coordinates (corresponding to the width of the coverslip), 2) randomized horizontal coordinates (corresponding to the length of the coverslip) and 3) randomized mixed (corresponding to randomized combinations of vertical and horizontal coordinates). For each sampling method, a minimum of 500 pollen grains was counted per slide. Pollen stainability was calculated as the percentage of viable pollen grains of total pollen grains counted. The methods were compared for statistical differences using the GLM procedure in SAS software version 8.1 (SAS Institute Inc., 2001). An ANOVA was conducted on normal data recorded as the proportion of viable pollen.

Mating system

Mating system studies were conducted to test for asexual seed production, self-compatibility, self-pollination, and cross pollination in the Maple River population,

which is the population with the highest level of fertile pollen and seed set. For these tests, twenty-two five-inch stolons, presumed to represent different genets, were collected from the Maple River population and grown in the greenhouse under a 16-18 day length at 70°C. The stolons exhibited vigorous growth in the greenhouse and were contained in 8-inch pots in flats of water. They were watered daily with fertilized water and treated with pesticides and fungicides as needed.

The following treatments were applied to ten randomly chosen plants: 1) unmanipulated, 2) emasculated, 3) self-pollinated and 4) cross-pollinated (Table 1). Self-compatibility is required for self-pollination but to understand the breeding system, the two must be distinguished. Seed set after treatments 1 and 3 both suggest self-compatibility. Seed set after treatment one further suggests the ability to self-pollinate.

Table 1: Experimental treatments for determining the mating system of Michigan monkey-flower. The outcome determined by each treatment is indicated with positive cells.

Treatment	self-pollination	self-compatibility	asexual reproduction	cross-pollination
1) unmanipulated	+	+	-	-
2) emasculated	-	-	+	-
3) self-pollinated	-	+	-	-
4) cross-pollinated	-	-	-	+

Experimental plants were enclosed within a fine mesh to exclude pollinators. Each treatment was randomly applied to one flower on each of five haphazardly chosen stolons per plant (Figure 1), thus a total of 5 flowers per treatment per plant and 50 flowers per treatment were manipulated. All treated flowers were marked for later identification. Unmanipulated flowers (treatment 1) were marked and not manipulated.

Emasculated and cross-pollinated flowers were emasculated prior to floral opening. Self-pollinated flowers were not emasculated. For self-pollinations and cross-pollinations, pollen was applied with a toothpick to the stigma of the experimental flower. Pollen was removed from the anthers of two or three flowers of non-experimental stolons per plant for self and cross pollen donors (two plants per supposed genet). Five randomly chosen plants, which were not experimental plants, were chosen as cross pollen donors and a mixture of pollen from the five plants was used in all cross-pollinations. The presence or absence of fruit set was recorded.

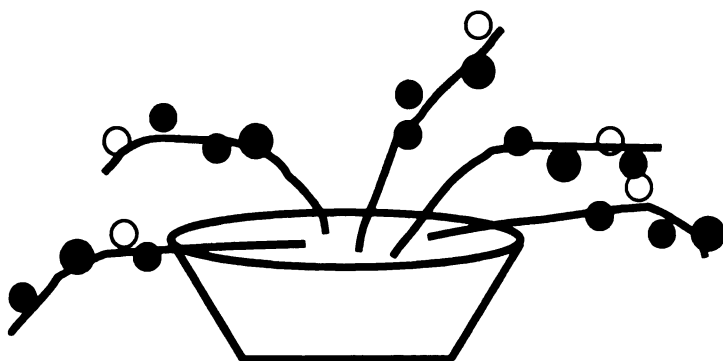


Figure 1: Assignment of mating system treatments to stolons of potted individuals. Each different coded circle represents one of the four treatments applied to one flower on each of five stolons: 1) Unmanipulated, 2) Emasculated, 3) Self-pollinated and 4) Cross-pollinated. Each plant produced many stolons and only treated stolons are depicted in the figure.

Interpopulation cross pollinations were conducted between Maple River individuals and pollen-sterile individuals to determine the ability of pollen-sterile plants to set seed. Two plants from each of four pollen-sterile populations (Burt Lake, Carp Creek, Glen Lake, Reese's Swamp) were randomly chosen as experimental plants. Four randomly chosen Maple River plants, which were not experimental plants or pollen donors for the intrapopulation cross experiments, were chosen as pollen donors. Two to

five flowers per plant were pollinated with a mixture of pollen from the four Maple River plants using a toothpick applied to the stigma. The presence or absence of fruit set was recorded.

Seed Germination

Fifteen seeds per treatment were allowed to germinate under different light and temperature conditions for 4 treatments: ~23°C light/dark, ~23°C dark, 8°C light/dark and 8°C dark. These temperatures were chosen because they were readily available (room temperature and refrigerator) and they encompass the range of water temperatures at which Michigan monkey-flower is known to grow in nature. Seeds were collected and pooled from five individuals in the greenhouse for germination experiments. Seeds were placed in sealed petri plates on moistened filter paper. Petri plates were maintained at room temperature on a lab bench near a window (approximately 23°C) or in a refrigerator (8°C) with a clear, glass door to allow light exposure. For light/dark treatments, seeds were exposed to approximately 16 hours light per day. For dark treatments, petri plates were contained in closed boxes and did not receive any light. The percent germination was measured for each treatment over a 30 day period.

Results

Pollen Stainability

Total pollen stainability did not differ between the three methods of sampling pollen stainability ($p=0.6756$). An image taken from a light microscope is shown in Figure 2. Pollen stainability of all individuals from Burt Lake, Carp Creek, Glen Lake

and Reese's Swamp was 0% (Table 2). Pollen stainability varied among the five Maple River individuals from 27-52% (Table 2).

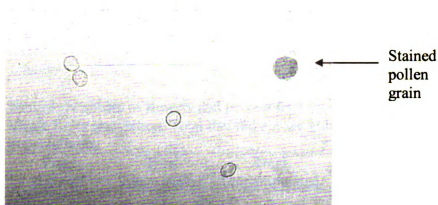


Figure 2: Image from a light microscope of pollen stained with aceto-carmin jelly. A stained pollen grain can be seen in the upper right corner of the slide. The other four grains are not stained.

Table 2: Pollen stainability of Michigan monkey-flower individuals sampled from five populations ($n > 500$ pollen grains for all samples).

Population	No. Individuals Sampled (No. Flowers)	Pollen Stainability (% stained)
Burt Lake	1 (1)	0
Carp Creek	5 (1)	0
Glen Lake	4 (1)	0
Maple River	5 (1)	27.4, 36.6, 46.0, 49.9, 51.6
Reese's Swamp	2 (1)	0

Mating System

Data for the intrapopulation Maple River crosses are reported in Table 3. A total of 50 flowers per treatment were pollinated, however, not all flowers survived to fruiting because of fungal disease and insect infestation in some plants. All surviving unmanipulated and self-pollinated flowers set fruit. No fruit set was observed in the

emasculated flowers. Of 35 surviving cross-pollinated flowers, three did not set fruit. For the interpopulational crosses between pollen-sterile individuals from other sites and Maple River individuals, 24 treated flowers representing all 4 pollen-sterile populations in the study survived to fruiting and all 24 set fruit (Table 4).

Table 3: Numbers of flowers and percent fruit set in crosses between plants from the Maple River population. Total that did not set fruit in cross-pollinated treatments shown in parentheses.

Plant Treated	Total Emasculated	Total Unmanipulated	Total Self-Pollinated	Total Cross-Pollinated
07A	4	3	3	4
09B	4	3	5	4
10A	5	4	4	4
12A	2	1	2	2
14A	3	4	2	3
28A	4	4	4	4
29A	5	5	5	5 (1)
31B	2	1	1	2
33B	4	4	4	4 (1)
34B	4	3	4	4 (1)
Total	37	32	34	36
% Setting Fruit	0%	100%	100%	91.7%

Table 4: Percentage fruit set between crosses of Maple River pollen donors and pollen-sterile pollen recipients.

Population	Number Flowers Treated	% Setting Fruit
Burt Lake	6	100%
Carp Creek	6	100%
Glen Lake	6	100%
Reese's Swamp	6	100%

Seed Germination

Percent germination for each treatment is shown in Table 5. After 7 days, ten of fifteen seeds had germinated at ~23°C in the light/dark treatment, one of fifteen seeds had germinated at ~23°C in the dark treatment, and no seeds germinated at 8°C in both the light/dark and dark treatments. No further germination was observed over the 30 day period.

Table 5: Percent germination for four treatments.

Treatment	Number Seeds Treated	% Germination
~23°C light/dark	15	66.7
~23°C dark	15	6.7
8°C light/dark	15	0
8°C dark	15	0

Discussion

Pollen Stainability

Earlier studies by Bliss (1983, 1986) have shown that pollen viability (measured as stainability) of Michigan monkey-flower is low in all populations compared to typical plant species. These results were consistent with those of Bliss. The Reese's Swamp population, which had not been tested for pollen viability prior to this study, had 0% viable pollen. Therefore, the only population known to have viable pollen is the Maple River population. Among the five individuals of the Maple River population tested, I found a two-fold variation in the levels of viability ranging from 27 to 52 percent.

Mating System

These results clearly show that individuals of Michigan monkey-flower are self-compatible, contrary to the results of Bliss (1983). Bliss initiated crossing experiments simultaneously with pollen measurements and crosses were conducted with pollen-sterile individuals only; she had no a priori knowledge that they were pollen-sterile. This result is consistent with reports of self-compatibility in other varieties of *Mimulus glabratus* (Vickery, 1991).

Individuals of Michigan monkey-flower do not produce seed asexually but those from the Maple River site are self-pollinating when grown in the greenhouse. Based on observations at the Maple River site (Bliss, 1986; A. Posto, pers. obs.), the amount of fruit set, based on the number of fruits per inflorescence per plant, due to self-pollination is higher in greenhouse grown Maple River plants than total observed fruit set in the field. Fruit set in nature may be limited by resource competition or, alternatively, handling the inflorescences in the greenhouse while labeling flowers or hand-pollinating other flowers on the same inflorescence could have caused pollen to be transferred to the pistil. However fruit set and seed set was observed on Maple River plants in the greenhouse that had not been handled, indicating that self-pollination is occurring. Reduced fruit set in cross-pollinated treatments is likely due to damage to the pistil during emasculation. The three flowers that did not set fruit were the first subjects of cross-pollination.

In interpopulational crosses in which Maple River individuals served as pollen donors for pollen-sterile individuals, 100% fruit set (as indicated by swelling of ovary and calyx) was observed, suggesting that the ovules are viable and will set seed.

However, in studies of synthesized F_1 hybrids of *Mimulus guttatus* and *M. luteus*, Roberts (1964) found that self-pollinated semisterile plants (1-26%, 12-31%, and 2-20% pollen viability) exhibited enlargement of the capsule and calyx, but no seed set. The same effect was observed in backcrosses between the hybrids and parents, but with a greater degree of enlargement. Roberts suggests this may be due to hormone action following pollination. Thus it is unclear whether fruit set in crosses between Maple River and pollen-sterile plants is due to seed development or possibly, hormone action.

Seed germination

There are no prior data on requirements for seed germination for Michigan monkey-flower. Although our results are preliminary, and from only a few individuals, germinating the seeds in light at room temperature (approximately 23°C) clearly produced the best results. This is notable because water temperatures at Michigan monkey-flower sites are considerably cooler than 23°C and full sunlight is generally lacking in their environment. Bliss (1983) found that water temperatures at eight populations ranged from 11-18°C (average is 14°C). My results suggest that natural seed germination in Michigan monkey-flower may be dependent on variability in water temperatures. Further support from additional experiments conducted across Michigan monkey-flower's natural temperature range is necessary.

Conclusions

Michigan monkey-flower is self-compatible and plants from the Maple River population are capable of self-pollination and regularly set selfed fruits in the greenhouse. There is considerable variation (27-52%) in pollen viability levels between individuals of the Maple River population, which has implications for mating, selection, and gene flow

within the Maple River population. The Reese's Swamp population, which had never before been studied, has 0% viable pollen, similar to other populations studied with the exception of the population at Maple River. Among four seed germination regimes tested, the highest rates of germination were observed at room temperature (approximately 23°C) under the light/dark treatment.

CHAPTER 3

THE TAXONOMIC ORIGIN OF MICHIGAN MONKEY-FLOWER

Introduction

Michigan monkey-flower, *Mimulus glabratus* var. *michiganensis* (Pennell) Fassett, is endemic to Michigan and is found within the Mackinac Straits and Grand Traverse regions of Michigan (Figure 3). It is a diploid perennial in a widespread and morphologically diverse complex of yellow monkey-flowers most commonly found in the western United States. Pennell (1935) originally described it as a subspecies of *M. glabratus*. Fassett (1939) proposed a change in rank to variety. Current workers treat the taxon as a variety (Bliss, 1983, 1986; Crispin and Penskar, 1989; Minc, 1989; Vickery, 1991; Voss, 1996). Michigan monkey-flower is of interest taxonomically because it has been suggested that it may have originated via hybridization (Bliss, 1983, 1986) and that it may merit promotion to specific status (Vickery, 1991).

Michigan monkey-flower is characterized by having a yellow, bilabiate corolla with an irregularly red-spotted lower lip. The leaves are opposite, ovate to broadly rounded with dentate margins and the lower leaves tend to have a well-developed petiole. The calyx is cup shaped with unequal teeth and becomes inflated at maturity. The fruit is a dehiscent capsule and the seeds are ovate with longitudinal striations.

Mimulus glabratus is the most widely distributed species in the genus *Mimulus* (Grant, 1924) and seven varieties of *M. glabratus* have been recognized (Grant, 1924; Pennell, 1935; Fassett, 1939; Skottsberg, 1953; Bliss, 1983). *Mimulus glabratus* var. *jamesii* (Benth.) A. Gray, James' monkey-flower, is the most broadly distributed variety

in the species ranging from western Quebec Province to Saskatchewan Province and south to Mexico. This variety is broadly distributed throughout in North America and is found throughout Michigan (Figure 3). Two other varieties occur in N. America: *M. glabratus* var. *utahensis* Pennell occurs from Colorado to Nevada and *M. glabratus* var. *oklahomensis* Fassett is found in Oklahoma, Nebraska and Kansas. *M. glabratus* HBK var. *glabratus* occurs throughout Mexico and Guatemala. The remaining two varieties are only found in S. America. *M. glabratus* var. *parviflorus* (Lindl.) Grant is found from Peru to Chile and Argentina and *M. glabratus* var. *externus* (Skottsb.) Skottsb. occurs only in the Juan Fernandez Islands.

Alam and Vickery (1973) and Vickery (1978) studied the interfertility of the *M. glabratus* complex. They focused on crosses between taxa with differing ploidy levels. There are three diploid varieties (vars. *michiganensis*, *oklahomensis*, and *utahensis*), one aneuploid tetraploid (var. *glabratus*), and two hexaploids (vars. *parviflorus* and *externus*). *Mimulus glabratus* var. *jamesii* has been reported to have both diploid and tetraploid individuals. There is complete reproductive isolation between all heteroploid levels (diploid, tetraploid, aneuploid tetraploid, and hexaploid; Alam and Vickery, 1973). Vickery (1978) found that none of the North American diploid varieties were completely reproductively isolated, based on their inter-fertility when crossed. His study of the inter-fertility *M. glabratus* varieties excluded Michigan monkey-flower, *M. glabratus* var. *michiganensis*.

Mimulus glabratus is included in section *Simiolus* of *Mimulus*. This section of approximately 20 species (Grant, 1924) includes *M. guttatus* DC, the Common monkey-flower, which is distributed from the Aleutian Islands of Alaska south to Mexico and in

the western US from California to the Rocky Mountains. Common monkey-flower is also found in Michigan (Figure 3). It was discovered in 1987 and is found at only one known location in the western Upper Peninsula, an area known for the occurrence of disjuncts of western species (Voss, 1996; USFWS, 1997).

There is considerable morphological similarity between Michigan monkey-flower, James' monkey-flower and Common monkey-flower. James' monkey-flower is characterized by having a smaller corolla than Michigan monkey-flower, few or no red spots on the lower lip, and a low, creeping habit. Common monkey-flower is characterized by having a larger corolla, a strongly red-spotted lower lip and an upright growth habit. The similarities between these two taxa and Michigan monkey-flower prompted two quantitative morphological studies, one by Bliss (1983, 1986) and the other by Minc (1989).

Bliss (1983, 1986) investigated the morphological similarity of Michigan monkey-flower and James' monkey-flower. Michigan monkey-flower was significantly larger for 22 of 25 quantitative floral and vegetative characters, with little overlap between taxa in floral measurements. In a study of all three taxa, Minc (1989) showed that Michigan monkey-flower is morphologically intermediate. In an analysis of seven floral characters, Michigan monkey-flower was significantly larger than James' monkey-flower for all characters and significantly smaller than Common monkey-flower for six characters. Minc found little overlap between Michigan monkey-flower and James' monkey-flower, but some overlap between Michigan monkey-flower and Common monkey-flower due to extensive variability within Common monkey-flower. There was a clear separation of the three taxa based on a canonical discriminant function analysis of

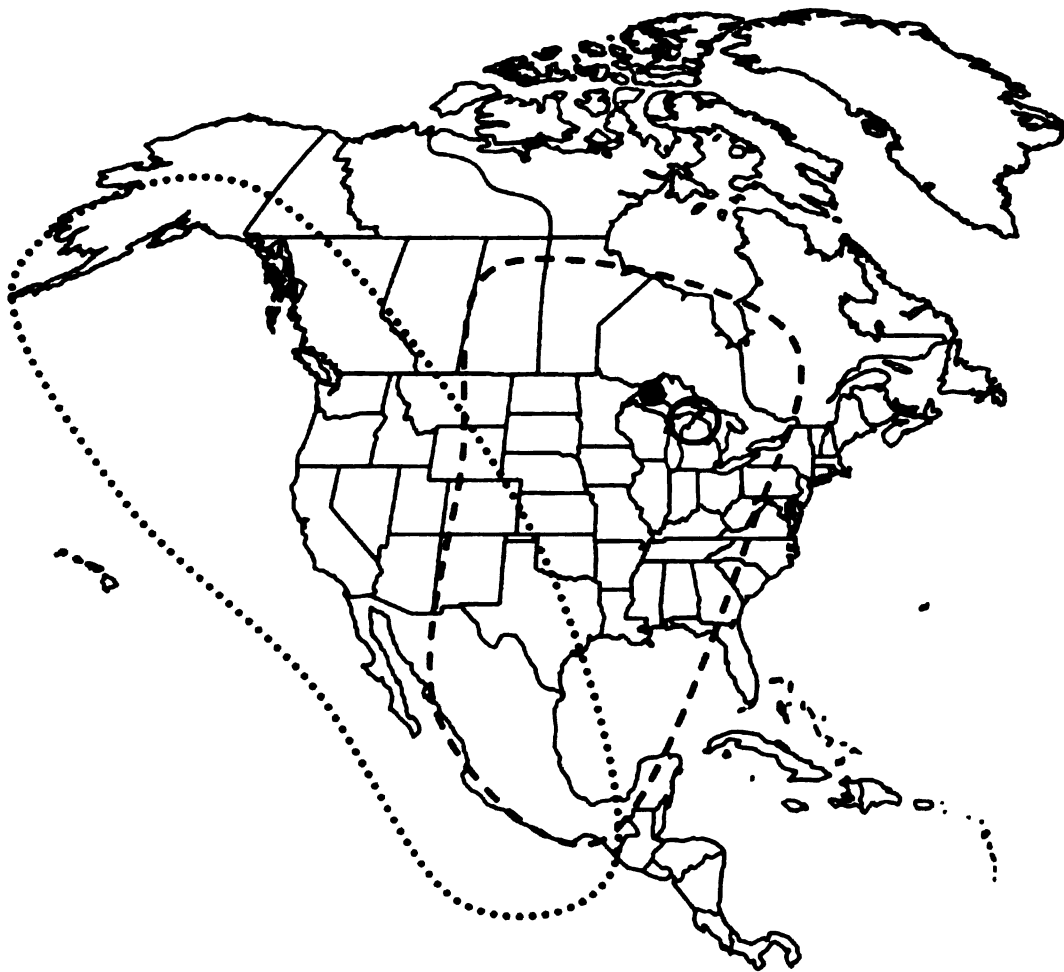


Figure 3: Distribution of Michigan monkey-flower (solid line), James' monkey-flower (dashed line) and Common monkey-flower (dotted line and filled circle in the western Upper Peninsula of Michigan) in North America.

two variates in which the first variate reflected differences in floral size and the second reflected the relative differences in ovary size.

Thus, Bliss and Minc demonstrated the morphological distinctiveness of Michigan monkey-flower from James' monkey-flower and Common monkey-flower. Furthermore, crossing experiments performed by Vickery (1991), using pollen-fertile individuals of Michigan monkey-flower, showed that Michigan monkey-flower was

completely reproductively isolated from James' monkey-flower and Utah monkey-flower, *M. glabratus* var. *utahensis*. In addition an allozyme analysis (Vickery, 1990) showed Michigan monkey-flower to be as distinct from the diploid as they are from the tetraploid and hexaploid forms of *M. glabratus* (Vickery, 1991).

Based primarily on morphological and cytological information, Bliss (1983, 1986) proposed three possible origins for Michigan monkey-flower: (1) Michigan monkey-flower (n=14,15) originated from a hybridization event between James' monkey-flower (n=14) and the Common monkey-flower (n=15), (2) Michigan monkey-flower originated from a chromosomal rearrangement of James' monkey-flower, or (3) Michigan monkey-flower originated as a disjunct aneuploid of the Common monkey-flower.

A number of factors are important when considering the likelihood of any of the possible hypotheses of origin for Michigan monkey-flower. The first hypothesis, that Michigan monkey-flower is a hybrid between James' monkey-flower and Common monkey-flower, is supported by intermediate morphology and low pollen viability in Michigan monkey-flower, characteristics usually associated with hybridization (Grant, 1981; Avise, 1994). However, hybridization between James' monkey-flower and Common monkey-flower seems unlikely for the following reasons: James' monkey-flower and the Common monkey-flower are not currently sympatric in Michigan and pollen flow over long distances seems unlikely. Furthermore, James' monkey-flower is primarily selfing and hybridization could only occur via pollination by animals. Because of the large difference in floral size between James' monkey-flower and Common monkey-flower, it is unlikely that the same pollinator species could effectively transfer

pollen between species. Also, Common monkey-flower may be a recent introduction to Michigan (Voss, 1996; USFWS, 1997).

Arguments pro and con can likewise be made for the hypothesis that Michigan monkey-flower arose from a chromosomal rearrangement of James' monkey-flower. Chromosomal rearrangements usually result in low fertility and morphological changes (Avisé, 1994; Rieseberg, 1997), as seen in Michigan monkey-flower. Cytological abnormalities cited by Tai and Vickery (1970, 1972) and Vickery (1978) for the *M. glabratus* complex corroborate the possibility of this origin (USFWS, 1997). On the other hand, the intermediate morphology of Michigan monkey-flower is unexpected if Michigan monkey-flower is derived solely from James' monkey-flower.

Arguments pro and con also exist for the third hypothesis, that Michigan monkey-flower originated as a disjunct aneuploid of the Common monkey-flower. Aneuploidy is known among monkey-flowers (Vickery et al., 1968) and the presence of Common monkey-flower in Michigan, which was undocumented when Bliss first proposed these hypotheses (Bliss, 1983), suggests that the disjunction event would not have had to occur across long distances. Neither low fertility nor intermediate morphology is expected with aneuploidy, and it is highly improbable that both occurred simultaneously by chance.

To address these hypotheses, the random amplified polymorphic DNA (RAPD) technique (Welsh and McClelland, 1990; Williams et al., 1990) was used to investigate the origin of Michigan monkey-flower. RAPD is based on the polymerase chain reaction (PCR) and utilizes random 10 base-pair primers to amplify fragments of total genomic DNA. The amplified DNA fragments are separated by gel electrophoresis and viewed as bands, which are scored for presence and absence. RAPD bands are genetic markers that

exhibit dominant inheritance, meaning band absence indicates a homozygous recessive genotype, but band presence does not distinguish between a homozygous or heterozygous genotype for band presence.

Patterns of band sharing between the taxa can be examined in order to discriminate between the hypotheses proposed by Bliss (1986). If Michigan monkey-flower originated via hybridization, the RAPD pattern should show an additive banding pattern for genetic markers of James' monkey-flower and Common monkey-flower. Likewise, if Michigan monkey-flower originated from James' monkey-flower or from Common monkey-flower, it should share bands with only the respective parent or bands from one parent and few unique bands. If Michigan monkey-flower is a derivative of either James' monkey-flower or Common monkey-flower it should not share any bands with the non-parental species unless the band is common to all three taxa.

RAPD is a useful technique because it offers an essentially unlimited number of markers for study, requires minimal amounts of DNA, is not limited to functional proteins, does not require sequence knowledge, and is relatively inexpensive (Hadrys et al., 1992; Williams et al., 1993). Furthermore, RAPD markers were appropriate for this study because they require very little plant material, which is a consideration when studying endangered species.

Materials and Methods

Sampling

Forty-two samples were included in the analysis including thirty-three samples of *Mimulus glabratus* and nine samples of *Mimulus guttatus* (Table 6). Sampling within

Mimulus glabratus included 19 samples of Michigan monkey-flower (*Mimulus glabratus* var. *michiganensis*) from 10 of 15 known populations. These samples were collected from across its range in Michigan. Sampling of other *M. glabratus* included 13 samples of James' monkey-flower (*Mimulus glabratus* var. *jamesii*) and one sample of Utah monkey-flower (*Mimulus glabratus* var. *utahensis*). Sampling was concentrated in James' monkey-flower because it is the only other variety of *M. glabratus* found in Michigan. Samples of James' monkey-flower are included from Michigan, Quebec, Oklahoma, Nebraska, Texas and Mexico. Samples of Common monkey-flower cover its geographic range and are from Michigan, California, Utah and Mexico. Sampling of Michigan populations of James' and Common monkey-flower concentrated on Michigan populations because local populations are more likely to have been important in the origin of Michigan monkey-flower.

DNA Isolation

Genomic DNA was isolated using the method of Doyle & Doyle (1987) as modified by Loockerman and Jansen (1996) for small amounts of plant tissue. DNA was isolated from fresh tissue or tissue preserved in silica or liquid nitrogen. Approximately 20 mg of fresh tissue or 1 mg dried tissue was ground in 0.4 mL extraction buffer consisting of 2% hexadecyltrimethyl-ammonium bromide (CTAB), 1.4 M NaCl, 25 mM EDTA , 100 mM Tris-HCl, pH 8.0, 0.5% β -mercaptoethanol and 4% polyvinyl-pyrrolidone (PVP-40). An additional 0.4 mL extraction buffer was added to the homogenate and incubated at 60°C for 20-30 minutes. DNA was extracted from the homogenate with the addition of two-thirds volume chloroform:octanol (24:1) followed by high-speed centrifugation. The resulting supernatant was transferred to a clean tube

Table 6: Identity of *Mimulus* samples in the RAPD analysis. Posto, Prather and Trull vouchers housed at MSC. RSA vouchers housed at RSA. Vickery vouchers housed at UT.

Taxa	ID No.	Location	Voucher
<i>M. glabratus</i> var. <i>michiganensis</i>	mich 1, 2	Benzie Co , MI	A.L. Posto 8
	mich 3, 4	Charlevoix Co., MI	A.L. Posto 11
	mich 5, 6	Cheboygan Co., MI	A.L. Posto 4
	mich 7, 8	Cheboygan Co., MI	A.L. Posto 7
	mich 9, 10	Emmett Co., MI	A.L. Posto 18
	mich 11, 12	Emmett Co., MI	A.L. Posto 13
	mich 13, 14	Leelanau Co., MI	A.L. Posto 6
	mich 15, 16	Leelanau Co., MI	A.L. Posto 9
	mich 17	Leelanau Co., MI	A.L. Posto 5
	mich 18, 19	Mackinac Co., MI	A.L. Posto 12
<i>M. glabratus</i> var. <i>jamesii</i>	jamsMI 1, 2, 3	Ontonagon Co., MI	S. J. Trull 336
	jamsMI 4, 5, 6	Ostego Co., MI	A.L. Posto 1
	jamsMI 7	Ostego Co., MI	A.L. Posto 10
	jamsMX 1	Chihuahua, Mexico	R.K. Vickery Jr. 12183
	jamsMX 2	Guanajuato, Mexico	R.K. Vickery Jr. 6201
	jamsNE	Custer Co., NE	R.K. Vickery Jr. 7135
	jamsOK	Woodward Co., OK	R.K. Vickery Jr. 7132
	jamsQE	Quebec, Canada	R.K. Vickery Jr. 10226
	jamsTX	TX	L.A. Prather 1805
<i>M. glabratus</i> var. <i>utahensis</i>	utah	Wayne Co., UT	R.K. Vickery Jr. 5265
<i>M. guttatus</i>	guttCA 1	San Bernardino Co., CA	RSA 20448
	guttCA 2	Los Angeles Co., CA	RSA 19998
	guttCA 3	Contra Costalo Co., CA	R.K. Vickery Jr. 5052
	guttMI 1, 2 & 3	Ontonagon Co., MI	S.J. Trull 332
	guttMX	Chihuahau, Mexico	R.K. Vickery Jr. 12180
	guttUT 1, 2	UT	A.L. Posto 3

and DNA was precipitated with two-thirds volume ice-cold isopropanol and stored at -20°C overnight. Precipitated DNA was condensed to a pellet in a centrifuge at high speed and washed with 0.8 mL 76% EtOH/0.01 M NH_4OAc . DNA was resuspended in water. DNA was quantified following extraction and was not cleaned prior to amplification.

DNA Amplification and Visualization

Samples were screened with 50 RAPD primers from sets A, B, and C from Operon Technologies (Alameda, CA). Primers that were easily amplified and which maximized the number of bands per primer were chosen. Reactions were carried out in 25 μl consisting of 1X *Taq* DNA polymerase buffer, 2 mM MgCl_2 , 0.2 mM of each dATP, dCTP, dGTP and dTTP (Boehringer Mannheim), 0.4 μM of primer (Operon), 1 unit of *Taq* DNA polymerase (Promega) and 25 ng of DNA. Amplifications were performed in an MJ Research Programmable Thermal Controller with the following PCR profile: 5 minutes at 94°C ; 45 cycles of 1 minute at 94°C , 1 minute at 75°C , 2 minutes at 35°C ; 5 minutes at 72°C ; 15°C soak. Each sample was amplified twice with each primer to demonstrate repeatability. The bands were resolved by electrophoresis on a 2% agarose gel in 1X TAE (Tris-Acetate-Borate) buffer, stained with ethidium bromide and visualized with UV illumination. Images were recorded digitally using AlphaImager 2000 software (Alpha Innotech).

RAPD Analysis

A schematic diagram of the similarity analysis is shown in Figure 4. RAPD images were scored for band presence and absence using Pro-RFLP Molecular Weight software (DNA Pro-Scan). Only reproducible bands were scored and reproducibility was

tested by scoring a minimum of two amplifications of each DNA sample with primer. A sample by sample similarity matrix (Appendix B) was constructed from a sample by marker data matrix (Appendix A) using Jaccard's coefficient. Jaccard's coefficient estimates similarity for all pairwise comparisons based on the number of shared traits and omits negative matches (matches based on the absence of a marker) (Sokal and Sneath, 1963). UPGMA clustering analysis was used to analyze the similarity matrix to examine relationships between the samples. NTSYSpc version 2.1 (Applied Biostatistics, Inc.) was used for similarity and clustering analyses.

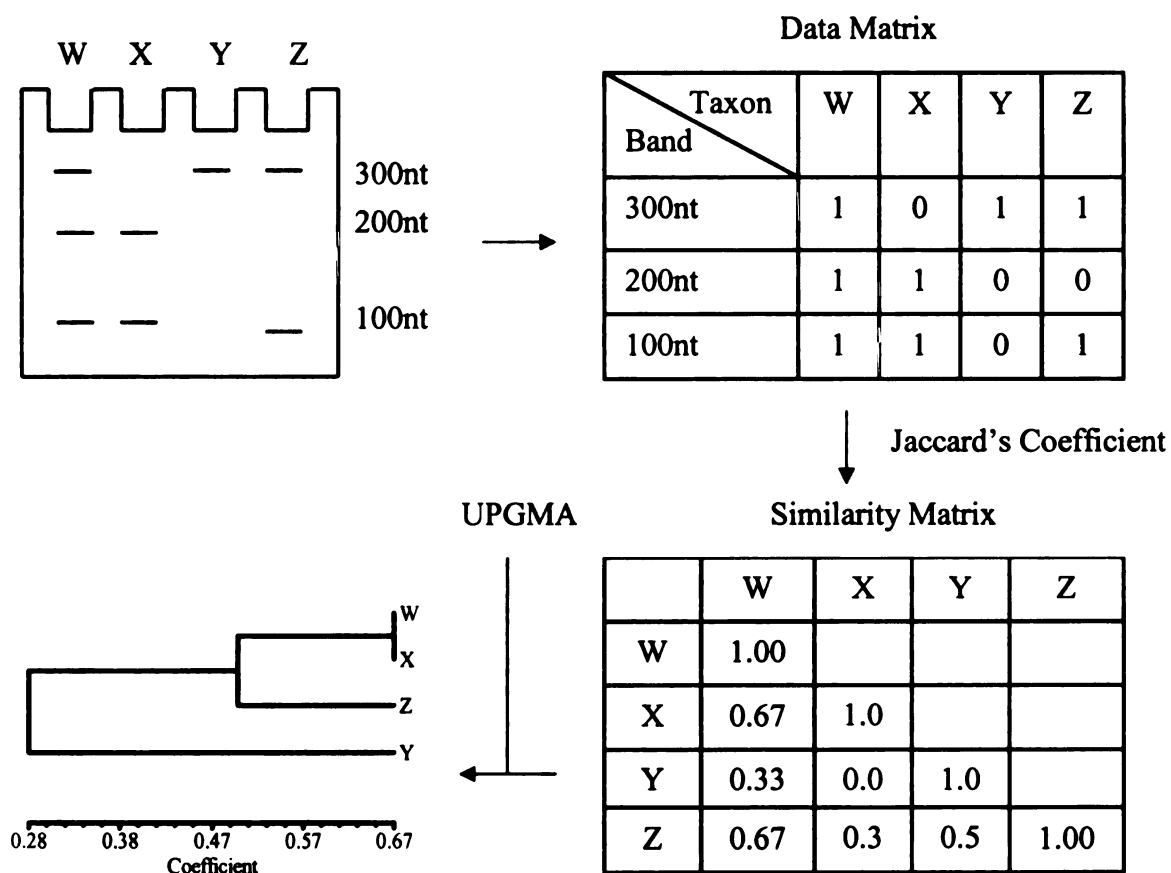


Figure 4: Similarity analysis flowchart. The gel image (upper left corner) is scored and the data is recorded in the data matrix. The similarity matrix is constructed using Jaccard's coefficient and a phenogram is constructed by clustering the similarity matrix using UPGMA.

Results

RAPD Primers

Six primers were chosen and a total of 99 amplified products were scored in the analysis (Table 7). The number of amplified products ranged from 8-22 products per primer. A representative image is shown in Figure 5.

Table 7: Primers used in the RAPD analysis.

Primer Identification	Nucleotide Sequence (5' to 3')	Number Amplified Products	Fragment Size Range (nt)
OPA-07	GAAACGGGTG	19	500-1760
OPA-11	CAATCGCCGT	22	350-1100
OPA-12	TCGGCGATAG	14	650-1550
OPB-05	TGCGCCCTTC	8	750-1325
OPB-10	CTGCTGGGAC	17	430-2000
OPC-02	GTGAGGCGTC	19	350-1325

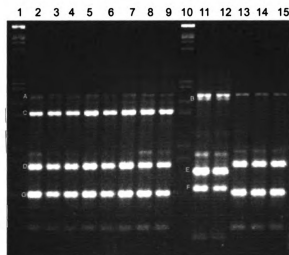


Figure 5: Image for thirteen individuals amplified with primer A07. Lanes 1 and 10 are molecular weight standards. Lanes 2-9 are Michigan monkey-flower, lanes 11-12 are Common monkey-flower and lanes 13-15 are James' monkey-flower. Band A is present in all taxa. Bands B, E and F are unique to Common monkey-flower. Band C is unique to Michigan monkey-flower. Bands D and G are shared by Michigan monkey-flower and James' monkey-flower.

RAPD Banding Patterns

Of the 99 amplified products, 70 were specific to individuals of one of the four taxa in the analysis (Table 8). The remaining twenty-nine bands are shared between samples of the four taxa.

Within Common monkey-flower, five polymorphic bands were found only in Michigan individuals, and 4 polymorphic bands were shared between individuals from Michigan and other localities.

Of the three bands Michigan and James' monkey-flower shared, one band was found in all individuals of Michigan monkey-flower and three of seven individuals of James' monkey-flower from Michigan populations. The second was found in all individuals of Michigan monkey-flower and all individuals of James' monkey-flower from Michigan and individuals from Texas and Quebec. The third was found in all individuals of Michigan monkey-flower and individuals of James' monkey-flower from all populations sampled (Mexico, Oklahoma, Nebraska, Texas and Canada).

Of the three bands Michigan -and Common monkey-flower shared, the first was shared between individuals of Michigan and Common monkey-flower from Michigan, Utah and California. The remaining two bands were shared between individuals of Michigan and Common monkey-flower from Michigan only.

The band shared between James' and Utah monkey-flower was found in individuals from all localities of James' monkey-flower (Michigan, Texas, Nebraska, Oklahoma, Quebec and Mexico).

For one polymorphic band shared between all four taxa, the band was absent in all samples of the Michigan populations of James' monkey-flower and Common monkey-

flower. Thus, all samples of Michigan monkey-flower shared this band with samples of James' monkey-flower from Nebraska, Oklahoma and Texas, with samples of Common monkey-flower from Utah, California and Mexico, and with Utah monkey-flower and not with local populations.

Table 8. Bands shared between taxa. Michigan monkey-flower is "MICH", James' monkey-flower is "JAMS", Utah monkey-flower is "UTAH", and Common monkey-flower is "GUTT". Fixed bands are found in all individuals of taxa in the left column, polymorphic bands are found in one or more, but not all, individuals. Moving down the table, bands shared between taxa are not cumulative. That is, the bands shared between all four taxa (MICH-JAMS-GUTT-UTAH) are not found in any other rows in the table. The same is true for all other rows.

Taxa (No. Individuals)	Band Frequency		Total
	Fixed	Polymorphic	
MICH-JAMS-GUTT-UTAH (42)	1	8	9
MICH-JAMS-GUTT (41)	0	3	3
MICH-JAMS-UTAH (33)	2	3	5
MICH-UTAH-GUTT (29)	0	1	1
JAMS-UTAH-GUTT (23)	0	1	1
MICH-JAMS (32)	0	3	3
MICH-GUTT (28)	0	3	3
JAMS-GUTT (22)	0	3	3
JAMS-UTAH (14)	0	1	1
MICH (19)	3	10	13
JAMS (13)	0	14	14
UTAH (1)	NA	3	3
GUTT (9)	0	40	40

Similarity Coefficients and UPGMA

Among the 19 samples of Michigan monkey-flower, eight different multilocus genotypes were represented (Figure 6). The average similarity coefficient among all Michigan monkey-flower individuals was 0.92, indicating high genetic similarity among

the samples (Table 9). Based on the UPGMA phenogram (Figure 6), all individuals of Michigan monkey-flower are distinct from the other taxa. Michigan monkey-flower exhibits much higher genetic similarity to James' monkey-flower (0.52) than it does to Common monkey-flower (0.24) (Table 9).

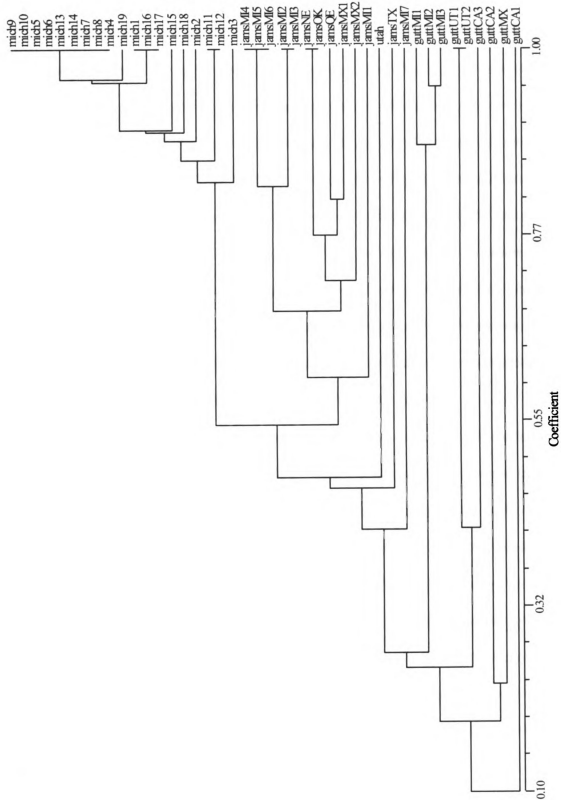
Among the 13 samples of James' monkey-flower, nine multilocus genotypes were represented. Utah monkey-flower falls within James' monkey-flower in the UPGMA phenogram and all individuals of *M. glabratus* (Michigan monkey-flower, James' monkey-flower and Utah monkey-flower) are distinct from Common monkey-flower, *M. guttatus* (Figure 6).

Among the nine Common monkey-flower samples eight multilocus genotypes were represented. All three individuals of Common monkey-flower from Michigan form a cluster with the samples of *Mimulus glabratus* and exhibit low genetic similarity to other individuals of Common monkey-flower (Figure 6).

Table 9: Genetic distance between Michigan monkey-flower, James' monkey-flower and Common monkey-flower. Except for within taxon comparisons, distance measures are calculated from the similarity between taxa and ignores similarity within taxa. (i.e. comparisons between michiganensis and guttatus are based only on the similarity between individuals of michiganensis and guttatus and not the similarity between individuals of michiganensis)

	Michigan monkey-flower	James' monkey-flower	Common monkey-flower
Michigan monkey-flower	0.92 (0.77-1.000)		
James' monkey-flower	0.52 (0.33-0.68)	0.66 (0.38-1.000)	
Common monkey-flower	0.24 (0.075-0.34)	0.20 (0.033-0.28)	0.30 (0.10-1.00)

Figure 6: UPGMA phenogram of 42 *Mimulus* samples genotyped by RAPD banding patterns. Taxon abbreviations follow those used in Table 3. Location abbreviations are as following: (CA = California, MX = Mexico, MI = Michigan, NE = Nebraska, OK = Oklahoma, QE = Quebec, UT = Utah), and ID number (refer to Table 6). The scale represents the similarity coefficient.



Discussion

The data presented in this analysis suggest that Michigan monkey-flower is not a recent hybrid of James' monkey-flower and Common monkey-flower. It is expected that a recent hybrid species should show additivity of parental marker alleles, but few if any unique alleles (Gallez and Gottlieb, 1982; Rieseberg et al., 1990; Wolfe and Elisens, 1995; Morrell and Rieseberg, 1998). Michigan monkey-flower exhibits an additive banding pattern for its putative parents because it shares as many bands with Common monkey-flower (that are not also shared with any other taxa) as it does with James' monkey-flower (Table 8). However, the number of shared bands is a small fraction of the total markers in the analysis and it is likely the sampling of other *M. glabratus* and *M. guttatus* in this analysis is insufficient to detect all possible genetic markers that might be shared between taxa. Thus the band sharing data is difficult to interpret.

In addition, Michigan monkey-flower possesses many unique genetic markers not found in any other taxa in the analysis (Table 8). In fact, it has nearly as many as found in the widespread James' monkey-flower (Table 8). Michigan monkey-flower is also unlikely to be of recent origin because it shares genetic markers with western populations of James' monkey-flower and Common monkey-flower that it does not share with more local, Michigan populations. Furthermore, Michigan monkey-flower is genetically distinct from other *M. glabratus* and *M. guttatus* in this study. It exhibits low genetic similarity to these other taxa and has a high intraspecific identity compared to the more widespread James' monkey-flower and Common monkey-flower (Michigan individuals of James' monkey-flower do not form a distinct cluster).

The relatively low genetic diversity between individuals of Michigan monkey-flower (average genetic similarity 0.92; range 0.77-1.0) does not contradict an ancient origin for this group. Speciation events are often associated with genetic bottlenecks (Grant, 1981; Mayr, 1954, 1963; Avise, 1994). Genetic diversity in Michigan monkey-flower is also expected to be low because asexual reproduction appears to predominate in all but one population (Bliss, 1983, 1986).

Evidence to support an ancient hybrid origin comes from the considerable genetic similarity between Michigan monkey-flower and Michigan populations of James' monkey-flower and Common monkey-flower. The average genetic similarity between Michigan monkey-flower and Michigan populations of James' monkey-flower is 0.76 versus 0.52 between Michigan monkey-flower to all samples of James' monkey-flower. The average genetic similarity between Michigan monkey-flower and Michigan populations of Common monkey-flower is 0.77 versus 0.24 between Michigan monkey-flower to all samples of Common monkey-flower. In addition, considerable divergence between Michigan populations and western populations of Common monkey-flower (Figure 6) suggest that Common monkey-flower in Michigan may be a natural population, and not a recent introduction. The potential existence of Common monkey-flower in Michigan for a long period of time bolsters the ancient hybridization hypothesis.

Based on the genetic distance between taxa it is likely that Michigan monkey-flower originated from James' monkey-flower. Michigan monkey-flower and James monkey-flower are much more similar to each other (0.52) than Michigan monkey-flower is to Common monkey-flower (0.24). Additionally James' monkey-flower and Common

monkey-flower are greatly differentiated (0.20). Based on these findings it seems more probable that Michigan monkey-flower originated from James' monkey-flower than Common monkey-flower or as a hybrid of James' and Common monkey-flower.

CHAPTER 4

CONCLUSION

My research on the biology of Michigan monkey-flower focused on its reproductive biology and taxonomic origin. An investigation of its reproductive biology specifically focused on its mating system parameters, pollen viability and seed germination. Experiments to determine the mating system of Michigan monkey-flower were conducted using individuals of the Maple River population, the only population known to have significant pollen viability and fruit set (Bliss, 1986). The results show that Michigan monkey-flower is self-compatible and plants from the Maple River population are capable of self-pollination and regularly set selfed fruits in the greenhouse.

Pollen viability was examined in five populations including the Maple River and Reese's Swamp populations. This study found considerable variation (27-52%) in pollen viability between individuals of the Maple River population. Maple River was reported to have 30% pollen viability (Bliss, 1986), but Bliss sampled only three individuals, one anther from each, from this population, thus variation between individuals, if detected, was not reported. Differences in pollen viability between individuals may have important consequences for reproduction and fitness among individuals of the population. These consequences in turn will have important implications for mating, selection, and gene flow within the Maple River population.

The Reese's Swamp population, which had not been previously studied, lacks viable pollen. Pollen viability was examined for individuals from Burt Lake, Carp Creek and Glen Lake and the results were similar to Bliss's results for these same populations. I

found that these populations lack viable pollen; Bliss found less than 1% viable pollen (1986). Thus the population at Maple River is remarkable for its production of viable pollen.

Among four seed germination regimes tested, the highest germination rates were observed at approximately 23°C with exposure to light. A significant decrease in germination was observed at approximately 23°C in the absence of light and no germination was observed at 8°C. Water temperatures at Michigan monkey-flower sites are considerably cooler than 23°C (Bliss, 1983) and full sunlight is generally lacking, therefore a reduction in germination at cooler temperatures and in the absence of light may have important consequences for recruitment of individuals from seed into the population.

Molecular markers were used in the taxonomic analysis to test between alternative hypotheses of hybrid origin from James' monkey-flower, *M. glabratus* var. *jamesii*, and Common monkey-flower, *M. guttatus*, versus divergence from one or the other. The presence of unique genetic markers in Michigan monkey-flower and its low genetic similarity to other *M. glabratus* and *M. guttatus* are inconsistent with a recent origin of Michigan monkey-flower. At this time it seems most likely that Michigan monkey-flower diverged from James' monkey-flower due to greater genetic similarity to it than to other taxa in the analysis. Among 19 samples of Michigan monkey-flower included in the taxonomic analysis, eight different multilocus genotypes were represented. Michigan monkey-flower is genetically distinct from the other taxa in the study and has relatively high intraspecific identity compared to the more widespread James' monkey-flower and Common monkey-flower.

Future work to clarify the origin of Michigan monkey-flower can be conducted through phylogenetic work and pollination crosses. The construction of a sectional phylogeny of *Simiolus* using representatives of other sections in *Mimulus* as well as groups outside of *Mimulus* as outgroups, may clear up relationship issues between *M. glabratus* and *M. guttatus* and the other species of the section. This data could be estimated from sequence data of nuclear and chloroplast genomes. Such a phylogeny is underway in Richard Olmstead's lab at the University of Washington, Seattle, WA.

Pollination crosses between Common monkey-flower and James' monkey-flower will determine if two species are interfertile and whether viable and/or fertile F₁ hybrids are produced. If hybrids could be formed it would be possible to determine morphological similarity of F₁ hybrids to Michigan monkey-flower by making quantitative observations of the F₁ hybrid morphology and conducting a comparative study to Michigan monkey-flower based on quantitative characters studied by Bliss (1983, 1986) and Minc (1989). Though extant populations of James' and Common monkey-flower may have diverged from ancient populations that could have contributed to a hybridization event leading to speciation, this information may provide evidence to support an ancient hybrid origin for Michigan monkey-flower. However, the inability to produce F₁ hybrids between crosses of James' monkey-flower and Common monkey-flower will not rule out an ancient hybrid origin.

APPENDICES

APPENDIX A
DATA MATRIX

APPENDIX A

	mich9	mich10	mich5	mich6	mich13	mich14	mich7	mich8	mich11	mich12	guttUT1	guttUT2	jamsM14	jamsM15
A07-1760	1	1	1	1	1	1	1	1	1	1	1	1	1	1
A07-1640	0	0	0	0	0	0	0	0	0	0	1	1	0	0
A07-1600	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-1598	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-1580	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-1515	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-1430	1	1	1	1	1	1	1	1	1	1	0	0	0	0
A07-1259	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-800	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-775	1	1	1	1	1	1	1	1	1	1	0	0	1	1
A07-765	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-750	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-740	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-700	0	0	0	0	0	0	0	0	0	0	1	1	0	0
A07-650	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-580	0	0	0	0	0	0	0	0	0	0	1	1	0	0
A07-560	1	1	1	1	1	1	1	1	1	1	0	0	1	1
A07-525	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-1100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-1075	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX A (cont'd.)

	mich9	mich10	mich5	mich6	mich13	mich14	mich7	mich8	mich11	mich12	guttUT1	guttUT2	jamsM14	jamsM15
A11-1030	1	1	1	1	1	1	1	1	1	1	1	1	0	0
A11-975	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-950	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-925	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-900	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-870	1	1	1	1	1	1	1	1	1	1	0	0	0	0
A11-860	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-845	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-820	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-800	1	1	1	1	1	1	1	1	0	0	0	0	1	1
A11-765	0	0	0	0	0	0	0	0	1	1	0	0	0	0
A11-750	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-737	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-625	0	0	0	0	0	0	0	0	0	0	0	0	1	1
A11-575	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-550	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-450	1	1	1	1	1	1	1	1	1	1	1	1	1	1
A11-415	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-350	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-1550	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX A (cont'd.)

	mich9	mich10	mich5	mich6	mich13	mich14	mich7	mich8	mich11	mich12	guttUT1	guttUT2	jamsM14	jamsM15
A12-1450	0	0	0	0	0	0	0	0	0	0	1	1	0	0
A12-1390	1	1	1	1	1	1	1	1	1	1	0	0	1	1
A12-1350	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-1315	0	0	0	0	0	0	0	0	0	0	1	1	0	0
A12-1290	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-1275	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-1100	1	1	1	1	1	1	1	1	1	1	1	1	1	1
A12-1025	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-850	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-750	0	0	0	0	0	0	0	0	0	0	1	1	0	0
A12-725	1	1	1	1	1	1	1	1	1	1	0	0	0	0
A12-700	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-650	1	1	1	1	1	1	1	1	1	1	0	0	1	1
B05-1325	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B05-1195	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B05-1145	1	1	1	1	1	1	1	1	1	1	0	0	1	1
B05-1075	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B05-1020	0	0	0	0	0	0	0	0	0	0	1	1	0	0
B05-950	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B05-845	1	1	1	1	1	1	1	1	1	1	0	0	1	1
B05-750	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX A (cont'd.)

	mich9	mich10	mich5	mich6	mich13	mich14	mich7	mich8	mich11	mich12	guttUT1	guttUT2	jamsM14	jamsM15
B10-2000	0	0	0	0	0	0	0	0	0	0	1	1	0	0
B10-1985	1	1	1	1	1	1	1	1	1	1	0	0	0	0
B10-1737	1	1	1	1	1	1	1	1	1	1	0	0	1	1
B101-635	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1465	0	0	0	0	0	0	0	0	0	0	1	1	0	0
B10-1430	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B10-1102	0	0	0	0	0	0	0	0	0	0	1	1	0	0
B10-1090	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1060	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1040	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-930	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-600	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-730	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-495	1	1	1	1	1	1	1	1	1	1	1	1	0	0
B10-430	1	1	1	1	1	1	1	1	1	1	0	0	1	1
C02-1325	0	0	0	0	0	0	0	0	0	0	1	1	0	0
C02-1300	1	1	1	1	1	1	1	1	1	1	0	0	0	0
C02-1200	0	0	0	0	0	0	0	0	0	0	0	0	1	1
C02-1100	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX A (cont'd.)

	mich9	mich10	mich5	mich6	mich13	mich14	mich7	mich8	mich11	mich12	gutUT1	gutUT2	jamsM14	jamsM15
C02-1010	1	1	1	1	1	1	1	1	1	1	0	0	1	1
C02-975	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-950	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-850	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-800	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-765	1	1	1	1	1	1	1	1	0	0	0	0	0	0
C02-755	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-730	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-700	0	0	0	0	0	0	0	0	0	0	1	1	0	0
C02-680	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-625	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-600	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-550	1	1	1	1	1	1	1	1	1	1	1	1	1	1
C02-500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-350	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX A (cont'd.)

	jamsMI6	mich1	mich2	mich15	mich16	mich3	mich4	mich18	mich19	mich17	jamsTX	jamsMI1	jamsMI2	jamsMI3
A07-1760	1	1	1	1	1	1	1	1	1	1	0	0	1	1
A07-1640	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-1600	0	0	0	0	0	0	0	0	0	0	1	0	0	0
A07-1598	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-1580	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-1515	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-1430	0	1	1	1	1	1	1	1	1	1	0	0	0	0
A07-1259	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-800	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-775	1	1	1	1	1	1	1	1	1	1	0	0	0	0
A07-765	0	0	0	0	0	0	0	0	0	0	1	1	1	1
A07-750	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-740	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-700	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-650	0	0	0	0	0	0	0	0	0	0	1	0	0	0
A07-580	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-560	1	1	1	1	1	1	1	1	1	1	0	1	1	1
A07-525	0	0	0	0	0	0	0	0	0	0	1	0	0	0
A07-500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-1100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-1075	0	0	0	0	0	0	0	0	0	0	0	1	1	1

APPENDIX A (cont'd.)

	jamsMI6	mich1	mich2	mich15	mich16	mich3	mich4	mich18	mich19	mich17	jamsTX	jamsMI1	jamsMI2	jamsMI3
A11-1030	0	1	1	1	1	1	1	1	1	1	0	0	0	0
A11-975	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-950	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-925	0	0	0	0	0	0	0	0	1	0	0	0	0	0
A11-900	0	0	0	0	0	0	0	0	0	0	0	1	1	1
A11-870	0	0	0	0	0	1	1	0	1	0	0	0	0	0
A11-860	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-845	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-820	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-800	1	1	1	1	1	1	1	1	1	1	0	1	1	1
A11-765	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-750	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-737	0	0	0	0	0	1	0	0	0	0	0	0	0	0
A11-625	1	0	0	0	0	0	0	0	0	0	0	1	1	1
A11-575	0	0	0	1	0	0	0	0	0	0	0	0	0	0
A11-550	0	0	0	0	0	0	0	0	0	0	1	0	0	0
A11-500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-450	1	1	1	1	1	1	1	1	1	1	1	1	1	1
A11-415	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-350	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-1550	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX A (cont'd.)

	jamsMI6	mich1	mich2	mich15	mich16	mich3	mich4	mich18	mich19	mich17	jamsTX	jamsMI1	jamsMI2	jamsMI3
A12-1450	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-1390	1	1	1	1	1	1	1	1	1	1	1	1	1	1
A12-1350	0	0	0	1	0	0	0	0	0	0	0	0	0	0
A12-1315	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-1290	0	0	0	0	0	1	0	0	0	0	0	0	0	0
A12-1275	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-1100	1	1	0	1	1	1	1	1	1	1	1	0	1	1
A12-1025	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-850	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-750	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-725	0	1	1	1	1	1	1	1	1	1	0	0	0	0
A12-700	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-650	1	1	1	1	1	1	1	1	1	1	1	0	1	1
B05-1325	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B05-1195	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B05-1145	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B05-1075	0	0	0	0	0	0	0	0	0	0	1	0	0	0
B05-1020	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B05-950	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B05-845	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B05-750	1	1	1	1	1	1	1	1	1	1	1	0	1	1

APPENDIX A (cont'd.)

	jamsMI6	mich1	mich2	mich15	mich16	mich3	mich4	mich18	mich19	mich17	jamsTX	jamsMI1	jamsMI2	jamsMI3
B10-2000	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1985	0	1	0	1	1	1	1	1	1	1	0	0	0	0
B10-1737	1	1	1	1	1	1	1	1	1	1	1	0	1	1
B101-635	0	0	0	0	0	0	0	0	0	0	1	0	0	0
B10-1500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1465	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1430	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B10-1102	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1090	0	0	0	0	0	1	0	0	0	0	0	0	0	0
B10-1060	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1040	0	0	0	0	0	0	0	0	0	0	1	0	0	0
B10-930	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-600	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-730	0	0	0	0	0	1	0	0	0	0	0	0	0	0
B10-495	0	1	1	1	1	1	1	1	1	1	1	0	0	0
B10-430	1	1	1	1	1	1	1	1	1	1	1	1	1	1
C02-1325	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-1300	0	1	1	1	1	1	1	1	1	1	0	0	0	0
C02-1200	1	0	0	0	0	0	0	0	0	0	0	1	1	1
C02-1100	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX A (cont'd.)

	jamsMI6	mich1	mich2	mich15	mich16	mich3	mich4	mich18	mich19	mich17	jamsTX	jamsMI1	jamsMI2	jamsMI3
C02-1010	1	1	1	1	1	1	1	1	1	1	1	1	1	1
C02-975	0	0	0	0	0	0	0	0	0	0	0	1	0	0
C02-950	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-850	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-800	0	0	0	0	0	0	0	0	0	0	1	0	0	0
C02-765	0	1	1	1	1	1	1	1	1	1	0	0	0	0
C02-755	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-730	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-700	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-680	0	0	0	0	0	0	0	1	0	0	0	0	0	0
C02-625	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-600	0	0	0	0	0	0	0	1	0	0	0	0	0	0
C02-550	1	1	1	1	1	1	1	1	1	1	1	1	1	1
C02-500	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-350	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX A (cont'd.)

	jamsMI7	guttMI1	guttMI2	guttMI3	jamsNE	jamsOK	guttCA1	guttCA2	guttMX	jamsQE	jamsMX1	utah	jamsMX2	guttCA3
A07-1760	0	0	0	0	0	0	0	0	0	0	0	0	0	1
A07-1640	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-1600	0	0	0	0	0	0	1	0	0	0	0	0	0	1
A07-1598	0	1	1	1	0	0	0	1	0	0	0	0	0	0
A07-1580	0	0	0	0	0	0	0	0	1	0	0	0	0	1
A07-1515	0	0	0	0	0	0	1	0	0	0	0	0	0	0
A07-1430	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-1259	0	0	0	0	0	0	0	1	0	0	0	0	0	0
A07-800	0	1	1	1	0	0	0	0	0	0	0	0	0	0
A07-775	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-765	1	0	0	0	1	1	0	0	0	1	1	1	1	0
A07-750	0	0	0	0	0	0	0	0	0	0	0	0	0	1
A07-740	0	0	0	0	0	0	0	0	0	0	0	0	0	1
A07-700	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-650	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-580	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-560	1	0	0	0	1	1	0	0	0	1	1	1	1	0
A07-525	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A07-500	0	0	0	0	0	0	0	0	0	0	0	0	1	0
A11-1100	0	0	1	1	0	0	0	0	0	0	0	0	0	0
A11-1075	0	0	0	0	0	0	0	0	0	1	1	0	0	0

APPENDIX A (cont'd.)

	jamsMI7	guttMI1	guttMI2	guttMI3	jamsNE	jamsOK	guttCA1	guttCA2	guttMX	jamsQE	jamsMX1	utah	jamsMX2	guttCA3
A11-1030	0	1	1	1	0	0	0	0	0	0	0	0	0	1
A11-975	0	0	0	0	0	0	0	0	0	0	0	0	0	1
A11-950	0	1	1	1	0	0	0	0	0	0	0	0	0	0
A11-925	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-900	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-870	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-860	0	0	0	0	0	0	0	1	0	0	0	0	0	0
A11-845	1	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-820	0	0	0	0	0	0	0	1	0	0	0	0	0	0
A11-800	1	0	0	0	0	0	0	0	0	1	1	1	1	0
A11-765	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-750	0	0	0	0	0	0	0	0	0	0	0	1	0	0
A11-737	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-625	0	0	0	0	1	1	0	0	0	1	1	0	0	0
A11-575	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11-550	1	0	0	1	1	1	1	0	0	0	1	1	1	0
A11-500	0	0	0	0	0	0	1	0	0	0	0	0	0	0
A11-450	0	1	1	1	1	1	0	0	0	1	0	1	0	1
A11-415	0	0	0	0	0	0	0	1	0	0	0	0	0	0
A11-350	0	0	0	0	0	0	0	0	1	0	0	0	0	0
A12-1550	0	1	1	1	1	1	0	1	0	1	1	0	1	1

APPENDIX A (cont'd.)

	jamsMI7	guttMI1	guttMI2	guttMI3	jamsNE	jamsOK	guttCA1	guttCA2	guttMX	jamsQE	jamsMX1	utah	jamsMX2	guttCA3
A12-1450	0	0	0	0	0	0	1	0	1	0	0	0	0	1
A12-1390	0	0	0	0	1	1	0	1	0	1	1	1	1	0
A12-1350	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-1315	0	0	0	0	0	0	0	0	0	0	0	0	0	1
A12-1290	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-1275	0	0	0	0	0	0	1	0	0	0	0	0	0	0
A12-1100	0	0	0	0	1	1	0	0	0	1	1	0	1	1
A12-1025	0	0	0	0	0	0	0	0	0	0	0	1	0	0
A12-850	0	0	0	0	0	0	0	1	0	0	0	0	0	0
A12-750	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-725	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12-700	0	0	0	0	0	0	1	0	0	0	0	0	0	0
A12-650	0	1	1	1	1	1	0	1	1	1	1	1	1	1
B05-1325	0	0	0	0	0	0	0	0	1	0	0	0	0	0
B05-1195	0	1	1	1	0	0	0	0	0	0	0	0	0	0
B05-1145	1	0	0	0	0	0	0	0	0	1	0	0	0	0
B05-1075	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B05-1020	0	0	0	0	0	0	0	1	0	0	0	0	0	0
B05-950	1	1	1	1	1	1	0	0	0	1	1	1	1	0
B05-845	1	0	0	0	1	1	0	0	0	1	1	0	0	0
B05-750	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX A (cont'd.)

	jamsM17	gutM11	gutM12	gutM13	jamsNE	jamsOK	gutCA1	gutCA2	gutMX	jamsQE	jamsMX1	utah	jamsMX2	gutCA3
B10-2000	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1985	0	1	1	1	0	0	0	0	0	0	0	0	0	0
B10-1737	1	0	0	0	1	1	0	0	0	1	1	1	1	0
B101-635	0	0	0	0	0	0	1	0	0	0	0	0	0	0
B10-1500	0	1	1	1	0	0	0	0	0	0	0	0	0	0
B10-1465	0	0	0	0	0	0	0	1	0	0	0	0	0	1
B10-1430	0	0	0	0	0	0	0	0	1	0	0	0	0	0
B10-1160	1	1	1	1	1	1	0	0	1	1	1	1	1	0
B10-1102	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1090	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1060	1	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-1040	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-930	1	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-600	0	1	1	1	0	0	1	1	0	0	0	0	0	0
B10-730	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10-495	0	0	0	0	1	1	1	1	1	0	0	1	0	1
B10-430	1	0	0	0	1	1	0	0	0	1	1	1	1	0
C02-1325	0	1	1	1	0	0	0	0	0	0	0	0	0	0
C02-1300	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-1200	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-1100	1	1	1	1	1	1	0	1	1	1	1	1	1	1

APPENDIX A (cont'd.)

	jamsMI7	guttMI1	guttMI2	guttMI3	jamsNE	jamsOK	guttCA1	guttCA2	guttMX	jamsQE	jamsMX1	utah	jamsMX2	guttCA3
C02-1010	1	0	0	0	1	1	0	0	0	1	1	1	1	0
C02-975	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C02-950	0	0	0	0	0	0	0	1	0	0	0	0	0	0
C02-850	0	0	0	0	0	0	0	0	1	0	0	0	0	0
C02-800	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-765	0	1	1	1	0	0	0	0	0	0	0	1	0	0
C02-755	0	0	0	0	0	0	0	0	0	1	0	0	0	0
C02-730	0	0	1	1	0	0	0	1	1	0	0	0	0	0
C02-700	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C02-680	0	1	1	1	0	0	0	0	0	0	0	0	0	0
C02-625	0	0	0	0	0	0	0	0	0	0	0	1	0	0
C02-600	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C02-550	1	1	1	1	1	1	1	1	1	1	1	1	1	1
C02-500	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C02-350	0	0	0	0	0	0	0	1	0	0	0	0	0	0

APPENDIX B
SIMILARITY MATRIX

APPENDIX B

	mich9	mich10	mich5	mich6	mich13	mich14	mich7	mich8	mich11	mich12	guttUT1	guttUT2	jamsMI4	jamsMI5
mich9	1.000													
mich10	1.000	1.000												
mich5	1.000	1.000	1.000											
mich6	1.000	1.000	1.000	1.000										
mich13	1.000	1.000	1.000	1.000	1.000									
mich14	1.000	1.000	1.000	1.000	1.000	1.000								
mich7	1.000	1.000	1.000	1.000	1.000	1.000	1.000							
mich8	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000						
mich11	0.893	0.893	0.893	0.893	0.893	0.893	0.893	0.893	1.000					
mich12	0.893	0.893	0.893	0.893	0.893	0.893	0.893	0.893	1.000	1.000				
guttUT1	0.282	0.282	0.282	0.282	0.282	0.282	0.282	0.282	0.289	0.289	1.000			
guttUT2	0.282	0.282	0.282	0.282	0.282	0.282	0.282	0.282	0.289	0.289	1.000	1.000		
jamsMI4	0.655	0.655	0.655	0.655	0.655	0.655	0.655	0.655	0.621	0.621	0.257	0.257	1.000	
jamsMI5	0.655	0.655	0.655	0.655	0.655	0.655	0.655	0.655	0.621	0.621	0.257	0.257	1.000	1.000
jamsMI6	0.655	0.655	0.655	0.655	0.655	0.655	0.655	0.655	0.621	0.621	0.257	0.257	1.000	1.000
mich1	0.963	0.963	0.963	0.963	0.963	0.963	0.963	0.963	0.857	0.857	0.289	0.289	0.679	0.679
mich2	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.786	0.786	0.270	0.270	0.667	0.667
mich15	0.897	0.897	0.897	0.897	0.897	0.897	0.897	0.897	0.800	0.800	0.275	0.275	0.633	0.633
mich16	0.963	0.963	0.963	0.963	0.963	0.963	0.963	0.963	0.857	0.857	0.289	0.289	0.679	0.679
mich3	0.871	0.871	0.871	0.871	0.871	0.871	0.871	0.871	0.781	0.781	0.256	0.256	0.576	0.576
mich4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.893	0.893	0.282	0.282	0.655	0.655

APPENDIX B (cont'd.)

	mich9	mich10	mich5	mich6	mich13	mich14	mich7	mich8	mich11	mich12	gutUT1	gutUT2	jamsM14	jamsM15
mich18	0.897	0.897	0.897	0.897	0.897	0.897	0.897	0.897	0.800	0.800	0.275	0.275	0.633	0.633
mich19	0.964	0.964	0.964	0.964	0.964	0.964	0.964	0.964	0.862	0.862	0.275	0.275	0.633	0.633
mich17	0.963	0.963	0.963	0.963	0.963	0.963	0.963	0.963	0.857	0.857	0.289	0.289	0.679	0.679
jamsTX	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.457	0.457	0.231	0.231	0.484	0.484
jamsM11	0.394	0.394	0.394	0.394	0.394	0.394	0.394	0.394	0.364	0.364	0.167	0.167	0.600	0.600
jamsM12	0.563	0.563	0.563	0.563	0.563	0.563	0.563	0.563	0.531	0.531	0.243	0.243	0.833	0.833
jamsM13	0.563	0.563	0.563	0.563	0.563	0.563	0.563	0.563	0.531	0.531	0.243	0.243	0.833	0.833
jamsM17	0.375	0.375	0.375	0.375	0.375	0.375	0.375	0.375	0.344	0.344	0.143	0.143	0.462	0.462
gutM11	0.314	0.314	0.314	0.314	0.314	0.314	0.314	0.314	0.286	0.286	0.273	0.273	0.250	0.250
gutM12	0.297	0.297	0.297	0.297	0.297	0.297	0.297	0.297	0.270	0.270	0.257	0.257	0.235	0.235
gutM13	0.289	0.289	0.289	0.289	0.289	0.289	0.289	0.289	0.263	0.263	0.250	0.250	0.229	0.229
jamsNE	0.533	0.533	0.533	0.533	0.533	0.533	0.533	0.533	0.552	0.552	0.273	0.273	0.667	0.667
jamsOK	0.533	0.533	0.533	0.533	0.533	0.533	0.533	0.533	0.552	0.552	0.273	0.273	0.667	0.667
gutCA1	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.086	0.086	0.129	0.129	0.065	0.065
gutCA2	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.184	0.184	0.200	0.200	0.176	0.176
gutMX	0.176	0.176	0.176	0.176	0.176	0.176	0.176	0.176	0.182	0.182	0.200	0.200	0.172	0.172
jamsQE	0.548	0.548	0.548	0.548	0.548	0.548	0.548	0.548	0.516	0.516	0.222	0.222	0.750	0.750
jamsMX1	0.484	0.484	0.484	0.484	0.484	0.484	0.484	0.484	0.452	0.452	0.200	0.200	0.667	0.667
utah	0.469	0.469	0.469	0.469	0.469	0.469	0.469	0.469	0.394	0.394	0.194	0.194	0.464	0.464
jamsMX2	0.467	0.467	0.467	0.467	0.467	0.467	0.467	0.467	0.433	0.433	0.212	0.212	0.583	0.583
gutCA3	0.263	0.263	0.263	0.263	0.263	0.263	0.263	0.263	0.270	0.270	0.419	0.419	0.235	0.235

APPENDIX B (cont'd.)

	jamsMI6	mich1	mich2	mich15	mich16	mich3	mich4	mich18	mich19	mich17	jamsTX	jamsMI1	jamsMI2	jamsMI3
mich9														
mich10														
mich5														
mich6														
mich13														
mich14														
mich7														
mich8														
mich11														
mich12														
guttUT1														
guttUT2														
jamsMI4														
jamsMI5														
jamsMI6	1.000													
mich1	0.679	1.000												
mich2	0.667	0.923	1.000											
mich15	0.633	0.929	0.857	1.000										
mich16	0.679	1.000	0.923	0.929	1.000									
mich3	0.576	0.839	0.774	0.788	0.839	1.000								
mich4	0.655	0.963	0.889	0.897	0.963	0.871	1.000							

APPENDIX B (cont'd.)

mich18	jamsMI6	mich1	mich2	mich15	mich16	mich3	mich4	mich18	mich19	mich17	jamsTX	jamsMI1	jamsMI2	jamsMI3
	0.633	0.929	0.857	0.867	0.929	0.788	0.897	1.000						
mich19	0.633	0.929	0.857	0.867	0.929	0.844	0.964	0.867	1.000					
mich17	0.679	1.000	0.923	0.929	1.000	0.839	0.963	0.929	0.929	1.000				
jamsTX	0.484	0.457	0.441	0.432	0.457	0.400	0.444	0.432	0.432	0.457	1.000			
jamsMI1	0.600	0.406	0.433	0.382	0.406	0.351	0.394	0.382	0.382	0.406	0.375	1.000		
jamsMI2	0.833	0.581	0.567	0.545	0.581	0.500	0.563	0.545	0.545	0.581	0.500	0.750	1.000	
jamsMI3	0.833	0.581	0.567	0.545	0.581	0.500	0.563	0.545	0.545	0.581	0.500	0.750	1.000	1.000
jamsMI7	0.462	0.387	0.414	0.364	0.387	0.333	0.375	0.364	0.364	0.387	0.400	0.440	0.481	0.481
gutMI1	0.250	0.324	0.303	0.306	0.324	0.282	0.314	0.343	0.306	0.324	0.222	0.188	0.235	0.235
gutMI2	0.235	0.306	0.286	0.289	0.306	0.268	0.297	0.324	0.289	0.306	0.211	0.176	0.222	0.222
gutMI3	0.229	0.297	0.278	0.282	0.297	0.262	0.289	0.316	0.282	0.297	0.237	0.171	0.216	0.216
jamsNE	0.667	0.552	0.536	0.516	0.552	0.471	0.533	0.516	0.516	0.552	0.630	0.520	0.680	0.680
jamsOK	0.667	0.552	0.536	0.516	0.552	0.471	0.533	0.516	0.516	0.552	0.630	0.520	0.680	0.680
gutCA1	0.065	0.086	0.091	0.081	0.086	0.075	0.083	0.081	0.081	0.086	0.194	0.033	0.061	0.061
gutCA2	0.176	0.184	0.194	0.175	0.184	0.163	0.179	0.175	0.175	0.184	0.189	0.118	0.167	0.167
gutMX	0.172	0.182	0.194	0.171	0.182	0.158	0.176	0.171	0.171	0.182	0.188	0.103	0.161	0.161
jamsQE	0.750	0.567	0.552	0.531	0.567	0.486	0.548	0.531	0.531	0.567	0.533	0.667	0.833	0.833
jamsMX1	0.667	0.500	0.483	0.469	0.500	0.429	0.484	0.469	0.469	0.500	0.517	0.583	0.750	0.750
utah	0.464	0.484	0.517	0.455	0.484	0.417	0.469	0.455	0.455	0.484	0.452	0.393	0.483	0.483
jamsMX2	0.583	0.483	0.464	0.452	0.483	0.412	0.467	0.452	0.452	0.483	0.500	0.440	0.600	0.600
gutCA3	0.235	0.270	0.250	0.256	0.270	0.238	0.263	0.256	0.256	0.270	0.243	0.143	0.222	0.222

APPENDIX B (cont'd.)

	jamsM17	guttM11	gutM12	gutM13	jamsNE	jamsOK	gutCA1	gutCA2	gutMX	jamsQE	jamsMX1	utah	jamsMX2	gutCA3
mich9														
mich10														
mich5														
mich6														
mich13														
mich14														
mich7														
mich8														
mich11														
mich12														
gutUT1														
gutUT2														
jamsMI4														
jamsMI5														
jamsMI6														
mich1														
mich2														
mich15														
mich16														
mich3														
mich4														

APPENDIX B (cont'd.)

	jamsMI7	guttMI1	guttMI2	guttMI3	jamsNE	jamsOK	guttCA1	guttCA2	guttMX	jamsQE	jamsMX1	utah	jamsMX2	guttCA3
mich18														
mich19														
mich17														
jamsTX														
jamsMI1														
jamsMI2														
jamsMI3														
jamsMI7	1.000													
guttMI1	0.161	1.000												
guttMI2	0.152	0.905	1.000											
guttMI3	0.182	0.864	0.955	1.000										
jamsNE	0.500	0.267	0.250	0.281	1.000									
jamsOK	0.500	0.267	0.250	0.281	1.000	1.000								
guttCA1	0.115	0.107	0.100	0.133	0.148	0.148	1.000							
guttCA2	0.091	0.226	0.250	0.242	0.226	0.226	0.148	1.000						
guttMX	0.154	0.185	0.214	0.207	0.231	0.231	0.190	0.231	1.000					
jamsQE	0.520	0.250	0.235	0.229	0.739	0.739	0.065	0.176	0.172	1.000				
jamsMX1	0.565	0.226	0.212	0.242	0.810	0.810	0.107	0.188	0.185	0.818	1.000			
utah	0.480	0.258	0.242	0.273	0.625	0.625	0.143	0.182	0.222	0.519	0.560	1.000		
jamsMX2	0.545	0.241	0.226	0.258	0.714	0.714	0.115	0.200	0.200	0.652	0.800	0.609	1.000	
guttCA3	0.086	0.212	0.200	0.194	0.250	0.250	0.179	0.212	0.259	0.200	0.176	0.171	0.188	1.000

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