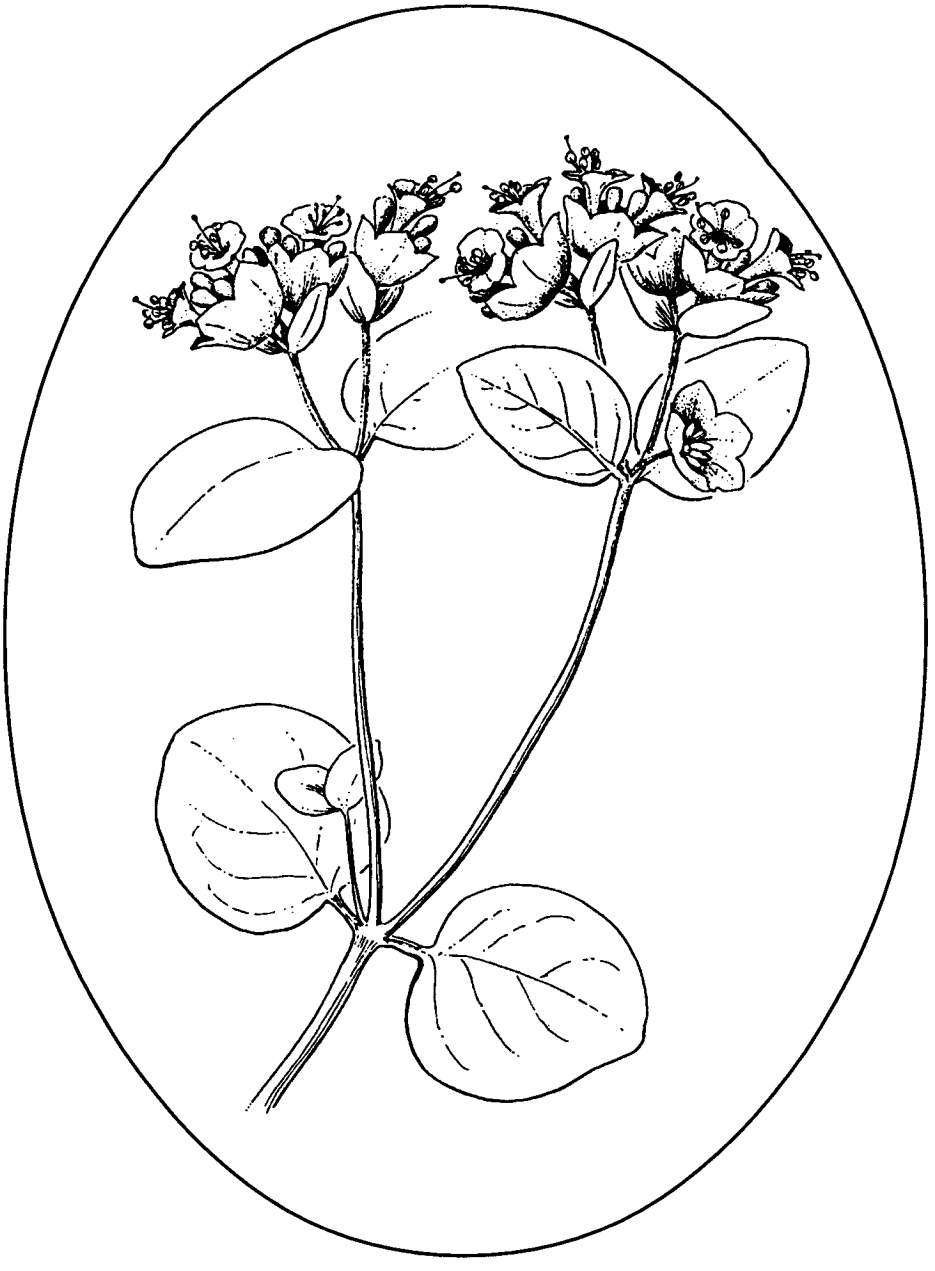
TECHNICAL BULLETIN NO. 97-17 IDAHO BUREAU OF LAND MANAGEMENT October 1997



Genetic Diversity, Gene Flow and Clonal Structure of the Salmon River Populations of Macfarlane's Four O'Clock

MIRABILIS MACFARLANEI (NYCTAGINACEAE)

by
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ABSTRACT

Many rare plant species occur in small, isolated populations. This can result in genetic drift and inbreeding which may lead to low genetic diversity. *Mirabilis macfarlanei*, a rare plant, is restricted to 19 relatively isolated populations along the Snake and Salmon River Canyons. We used enzyme electrophoresis to estimate genetic diversity, levels of gene flow, and clonal structure in eight selected populations of *M. macfarlanei* on the Salmon River. The analysis indicated that levels of genetic diversity were lower than values found with other plant allozyme data with similar life history traits reviewed in published papers. The measure of genetic differentiation among populations, F_{ST}, was 0.263 indicating low gene flow. We found high genetic similarity among populations using genetic distance values. Differentiation among populations was not closely related to the distances among populations. The clonal maps of Cody Draw, McKinzie Creek and Skookumchuck populations suggested diverse clonal growth forms with more widespread clones at Skookumchuck.

INTRODUCTION

Genetic diversity is thought to be an important predictor of the ecological success of a species or population (Beardmore, 1983; Holsinger and Gottlieb, 1991; Huenneke, 1991). Loss of genetic diversity may reduce a population's ability to adapt to changing environments, and increase its susceptibility to disease and pests (Barrett and Kohn, 1991). Because many rare plant species occur in small, isolated populations, they have an increased risk of losing valuable genetic variation due to inbreeding, genetic drift and reduced gene flow. Thus, preserving or augmenting genetic variation and gene flow are important considerations in conservation efforts.

The loss of genetic diversity in rare or small populations may result from genetic bottlenecks, random genetic drift, inbreeding or any combination of the three. Genetic bottlenecks result from a sudden large decrease in population size at a certain time or place. If individuals carrying rare genotypes are lost, then that rare genotype or allele may be lost from the population altogether. The evolutionary consequence of this loss may be a reduction in the number of alleles at certain loci, thereby reducing the number of polymorphic loci and genetic diversity (Barrett and Kohn, 1991).

Random genetic drift can have effects similar to a bottleneck on genetic diversity. Unlike a bottleneck, genetic drift does not entail a large population crash, but instead results from the random loss and fixation of alleles in finite populations over time. In natural populations this occurs through random sampling of gametes, and only a portion of genetic information is passed from parents to progeny. This can result in changes in allele frequency and/or loss of alleles from populations. The size of a population affects the degree to which genetic drift operates. Small

populations will lose alleles more rapidly than large populations, thus further reducing genetic diversity in a positive feedback loop.

Population size can also influence genetic diversity through potential inbreeding. Individuals in small and isolated populations are more likely to mate with close relatives. Frequent inbreeding increases homozygosity and may lead to inbreeding depression (Frankel and Soule, 1981; Ellstrand, 1992). Inbreeding can be further increased in self-compatible plants that are clonal and occur in large patches because pollinators are likely to move among ramets of the same genet (Handel, 1985; Ellstrand and Roose, 1987; Aspinwall and Christian, 1992).

The loss of genetic diversity can potentially be overcome by gene flow from other populations (Wright, 1931; Slatkin, 1985; Lacy, 1987). Wright (1931) suggested that the adverse effects of genetic drift can be reduced with the immigration of one or more individuals per generation into a population. Wright (1943) also proposed that gene flow will decrease with increased geographic distance between populations in his Isolation-by-Distance Model. Thus, greater genetic distinction would be expected for populations that are further apart. Limited gene flow among populations may cause greater population differentiation (Slatkin, 1985) and will also lower the genetic diversity of each population (Allendorf, 1983; Chesser, 1983). Therefore, it is of interest to estimate gene flow occurring among populations.

In the present study, we used enzyme electrophoresis to examine the population genetics of the rare plant, *Mirabilis macfarlanei* Constance and Rollins (Nyctaginaceae). MacFarlane's Four O'clock, *M. macfarlanei,* is listed as threatened under the Federal Endangered Species Act in the Federal Register. All nineteen known populations of *M. macfarlanei* exist within two counties: Idaho Co., Idaho and Wallowa Co., Oregon. Most populations are isolated by more

than 3 kilometers. These relatively small, isolated populations are found growing in the steep, basaltic canyons of the Snake, Salmon and Imnaha Rivers (Fig. 1). MacFarlane's Four O'clock is a long-lived perennial forb that reproduces sexually and clonally from a thick woody rhizome that sends out many shoots (collectively called a genet).

Current population estimates range from approximately 7,000 stems (ramets) at Long Gulch to 2 stems at WestKurry 2 (Craig Johnson, 1995 and personal observation). Seven of the 19 populations probably contain fewer than 150 stems. The size of the clones is not known but it is possible that smaller populations are comprised of only one or a few clones (one genet). Such small, isolated populations of MacFarlane's Four O'Clock are likely to have low genetic diversity in comparison to widespread species.

Population sizes vary greatly between years (Craig Johnson, BLM reports and personal observation). Some individuals appear to remain dormant during dryer years and grow only during years when adequate rainfall is received in the early spring (personal communication, Craig Johnson, BLM). Such fluctuations in population sizes can potentially act as bottlenecks during poor growth years. If some genets do not grow during bad years, seeds produced may have reduced numbers of alleles and lower genetic diversity.

We used starch gel electrophoresis to address the following objectives: 1) estimate the amount of genetic variability within populations; 2) measure the amount of genetic differentiation among populations; 3) estimate levels of gene flow among populations; and 4) provide a clonal map using multilocus enzyme markers to infer individual genotypes. The patterns of genetic diversity and gene flow for *M. macfarlanei* were then compared to published data on endemics with small, isolated populations and other clonal plants.

MATERIALS and METHODS

Sampling

Populations--Eight populations (Table 1) from the Salmon River in west central Idaho were selected for study (Maps 1 and 2). Two leaf samples were collected from each of approximately 40 ramets (stems) from each population during May of 1994. Leaves were stored on ice until arrival at the lab for analysis. The sampling schemes were as follows:

John Day and Long Gulch - Stems were randomly selected by traversing the populations and collecting leaves from stems that were more than 10m apart.

Slicker Bar - Stems were selected every 3m, from parallel transects across the top, middle and bottom of the population.

Cody Draw - The entire population was mapped and two leaves were collected from each stem.

Lucile Caves - This population has 3 planting sites established in 1988 by the BLM. Leaf samples were collected and mapped from stems found in sites 1 and 3.

Lucus Draw - This intermediate sized population was sampled from 15 clumps[1] that were ≥ 10m apart. Leaf samples were taken from two stems in each clump to determine if clumps were of a single clone. In addition, 10 individual stems were sampled.

McKinzie Cr. - All clumps in the population were sampled and mapped. Three stems were selected from each clump to determine if clumps were of a single clone.

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[1]A clump is defined as a group of stems growing collectively, with stems not separated by more than 30 cm from nearest neighbor. Clumps were separated from other clumps by 1 meter or more.

Skookumchuck - All individual stems in the 3'x 3' BLM monitoring plot #5 were mapped and sampled. All clumps or individual stems were sampled and mapped in the population outside of the plot.

We originally proposed to sample stems that were at least 10 meters apart to avoid sampling within the same clone. Because some of the populations were small, we were unable to follow this plan. Therefore, clonal maps were drawn for small populations and only those genotypes that appeared to be individual clones were entered into the population genetic analyses. For instance, 49 stems were sampled at Cody Draw but only the 11 individual clones that were identified were used for the population analysis.

Clonal mapping--To determine the number and distribution of genets and ramets within an area, clonal maps were constructed using multi-locus genotypes for the small populations: Cody Draw, McKinzie Creek and Skookumchuck. In addition, an 8m x 8m plot and 1.5 m x 130.65 m belt transect (5 ft x 435.5 ft) were mapped at Long Gulch. The plot was located 180 degrees off the start point (0 ft) of the BLM's belt transect number 2. All stems in the plot and along transect #2 were mapped on a grid and leaves were collected for analysis .

Electrophoretic analysis

Starch gel electrophoretic techniques followed those of Soltis et al. (1983). Leaf material was ground in a 0.10 M tris-HCl-PVP, pH 7.5 buffer with 0.1 % mercaptoethanol (Soltis et al. 1983). The ground material was soaked up with filter-paper wicks which were inserted into a 11.1% (w/v) starch gel (see Fig. 2). To find resolvable banding patterns, a preliminary test of twenty-one enzymes was conducted using various buffer systems (see Table 2). Ten polymorphic enzymes were found during the initial study (see Table 2). Of the twenty-one enzymes tested,

only AAT (1), ACON (3), IDH (1), LAP (1), MDH (3), PGI (1), PGM (2), SKDH (1) and TPI (3) had suitable resolution and dependability (number of loci for each enzyme in parenthesis). We used buffer system 8 to resolve the enzymes PGI, LAP, AAT and TPI, and buffer system 11 to resolve ACON, IDH, MDH, SKDH, and PGM. Staining for all enzymes followed recipes of Soltis et al. (1983). Photographs and drawings were made for each gel throughout the work.

Data analysis

Genetic Diversity--The electrophoretic data were analyzed using the computer program BIOSYS-1 (Swofford et al. 1981). Allele frequencies for each population and individual genotypes for each locus of each plant were entered into an input file. The data from the eight populations were used to generate the following measures of genetic diversity: 1) the percentage of loci that are polymorphic, 2) average number of alleles per locus, 3) average number of individuals sampled that are heterozygous at a locus (H_{dc}), 4) proportion of heterozygotes under Hardy-Weinberg expectations (HW_{exp}) and 5) number multilocus genotypes per population. Loci were considered polymorphic if the second allele was at a frequency above 5% in a population. The average number of heterozygotes or direct count is the number of heterozygotes observed for each locus divided by the number of individuals sampled and then averaged across all loci. H_{exp} is an overall indication of diversity reflecting the relative amount of polymorphisms occurring in the populations using Hardy-Weinberg proportions to determine expected number of heterozygotes.

The Hardy-Weinberg (HW) principle assumes that with random mating of diploid individuals the frequencies of the genotypes *AA*, *Aa* and *aa* are p^2, $2pq$ and q^2, respectively, where

p represents the allele frequency of A and q of a, and $p + q = 1$. Therefore the expected number of heterozygotes (Aa) is $2pq$ multiplied by the number individuals sampled.

Levels of Heterozygosity--Levels of heterozygosity can indicate how much random mating is occurring in populations. If plants are diploid and predominantly selfing, there should be fewer heterozygotes in the population than expected under HW. To determine whether there were low levels of heterozygotes, a fixation index (F) was calculated for each locus in each population. F is a simple comparison of the number of heterozygotes observed to the number expected under HW where $F=1 - (H_{obs}./H_{exp})$. If F is less than zero it indicates an excess of heterozygotes and if F is greater than zero there is a deficiency of heterozygotes. Significance was tested with a Chi-Square analysis.

Population Differentiation--A measure of genetic similarity between populations was determined by BIOSYS-1 using Nei's (1978) unbiased measure of genetic distance (D). Nei's genetic distance is based on difference in allele frequencies between pairs of populations and is defined as $D = -\ln [G_{XY}/ (G_X G_Y)^{1/2}]$ where G_X, G_Y and G_{XY} are the means of $\sum p^2_i$, $\sum q^2_i$ and $\sum p^2_i q^2_i$ over all loci examined. Thus if D=0, then populations have the same allele frequencies at each locus and populations are genetically very similar. However, if D is near 1, then populations are fixed for different alleles at all loci. A dendrogram was constructed using a UPGMA cluster analysis (Swofford et al. 1981) that depicts genetic similarities among populations.

To examine the effects of isolation on gene differentiation (Slatkin, 1993), Nei's genetic distance was also compared to geographic distance between populations using Pierson's correlation coeffictient (r) for each pair of populations. The correlation coefficient measures the

strength of linear association between the genetic distance and geographic distance to determine if greater differentiation occurs with increased geographic separation.

Allele frequency data were used to generate Wright's F-statistics, another measure of genetic differentiation (Wright, 1951). The distribution of genetic variation within and among the eight populations can be explained by Wright's F-statistics: F_{IS} and F_{ST}. F_{IS} measures deviation within populations from Hardy-Weinberg proportions averaged across populations. The deviation is estimated by : $F_{IS} = 1 - (H_{obs}/H_{exp})$ where H_{obs} is the actual number of individuals that are heterozygous at a particular locus divided by the number sampled, and H_{exp} is the number of heterozygotes expected under Hardy-Weinberg proportions. F_{IS} measures the reduction of heterozygosity within populations: zero indicates random mating and positive values indicate potential inbreeding.

The F_{ST} value correlates random gametes within subpopulations with respect to the total population, thus estimating the amount of genetic differentiation among populations. The most commonly used equation is

$$F_{ST} = s_p^2/p(1-p)$$

where s_p^2 is the variance in allele frequency among populations and p is the mean allele frequency. F_{ST} can vary between 1 and 0, where zero indicates there is no population differentiation and values near 1 indicate high population differentiation. The value can be tested as to whether it is significantly different than zero using a modified chi-square analysis used by Workman and Niswander (1970): $X^2 = 2NF_{ST}(k-1)$, with (k-1)(s-1) degrees of freedom, where k is the number of alleles, s is the number of populations analyzed and N is the number of samples used.

Gene Flow--The amount of differentiation among populations, F_{ST}, can be used to calculate an indirect estimate of gene flow among populations. Indirect measures of gene flow are obtained from F_{ST} using Wright's equation: $Nm = \{(1/F_{ST}) - 1\}/4$ where *Nm* is the number of individuals migrating per generation (Wright, 1943; Slatkin and Barton, 1989). An estimate of $Nm = 1$ indicates one migrant arrives every generation to a population. Values less than one are considered too low to counteract the effects of genetic drift (Slatkin, 1985).

RESULTS

Allozymes

Enzymes--From nine enzymes, sixteen loci were interpreted: *Acon-1, Acon-2, Acon-3, Aat-1, Mdh-1, Mdh-2, Mdh-3, Tpi-1, Tpi-2, Tpi-3, Skdh-1, Lap-1, Pgi-1, Pgm-1, Pgm-2* and *Idh-1* (see Fig. 3 for photos). When more than one locus (or isozyme) was observed per enzyme, the slowest locus was numbered *1* and faster loci were numbered sequentially. Likewise, polymorphic loci were labeled with *a* for the slowest allele and faster alleles were labeled alphabetically. Allele frequencies by locus for each population are given in Table 3.

Putative polysomic inheritance--During the course of the analysis, several loci displayed bands suggestive of polysomic inheritance (*Tpi-1, Tpi-3, Mdh-2, Mdh-3*) as described by Soltis and Rieseberg (1986). These loci had unequal banding intensity among polymorphic alleles. For instance, a heterozygote did not always have two equal bands, but sometimes had one dark band and one light band. This may indicate excess number of alleles, which could occur if multiple chromosomes were present. The remaining enzymes appeared to have normal disomic inheritance.

We investigated the possibility that *M. macfarlanei* was polyploid by counting chromosomes. We found n=33 with normal bivalents forming (Barnes and Windham, unpublished data). Pilz (1978) counted 33 chromosomes for 6 other species of *Mirabilis*. These results are above the n=13 criterion for demarcating polyploids as proposed by Stebbins (1971), Goldblatt (1980) and Grant (1981). Seven members of this genus have the same high chromosome count, suggesting that at least these members of the genus are paleopolyploids, rather than neopolyploids. Ancient polyploidy is further supported by the observation that some enzymes have polysomic and others disomic inheritance (Li, 1982; Wilson et al. 1983). Over time, diploid inheritance in polyploids can result from gene silencing, in which redundant genetic information is no longer transcribed (Werth and Windham 1991).

Polyploids are usually formed by the fusion of unreduced and reduced gametes (i.e., during meiosis 2n gametes are produced rather than 1n) (Harlan and DeWet, 1975). Such polyploids are produced in two steps, first from diploid to triploid (fusing of 2n and 1n gametes), and then from triploid to tetraploid. The triploid intermediate step is generally infertile but some unreduced 3n gametes are produced and join with 1n gametes to produce a viable tetraploid. Likewise, hexaploids and octaploids can be formed by further production of unreduced gametes and fertilization.

There are two main classifications of polyploids: allopolyploids and autopolyploids. Allopolyploids occur when two closely related species cross and form progeny that have the genome of both parents. For example, if parent A has n=15 chromosomes and parent B has n=13, the resulting progeny would have n=28. Allopolyploids exhibit diploid inheritance but usually have duplicate copies of each loci (isozymes) (Gottlieb, 1982). Enzyme electrophoresis banding

would show only two possible alleles for heterozygous individuals. Often, allopolyploids exhibit "fixed" heterozygosity as a result of the two parent crosses having different alleles fixed at a particular locus, the cross results in fixed heterozygosity (Soltis and Riesberg, 1986).

Autopolyploids are formed from a single species through the production and fusion of unreduced gametes. Because the genetic information is similar, pairing can occur among the duplicated chromosomes and loci can have more than 2 alleles per locus. This is termed polysomic inheritance. An autopolyploid usually has polysomic inheritance (i.e. hexaploids will have up to 6 alleles per locus and tetraploids will have up to 4 alleles per locus, rather than 2 alleles per locus as found in diploids and allopolyploids). An autopolyploid with a polymorphic locus having two alleles (A and B) can have (p + 1) different genotypes where p is the ploidy level. For example, a tetraploid would have 5 genotypes, a hexaploid would have 7 possible genotypes. Multiple alleles at one locus can be visualized with enzyme banding intensity. Genotypes can be determined for polymorphic loci based on the relative staining intensity of the enzyme bands. For example, a tetraploid individual with a genotype of AAAB would have darker banding at the A allele than at the B allele, because there were 3 copies of the A enzyme and 1 copy of the B enzyme in the plant

Much debate has revolved around the classifications and the adaptiveness of polyploids (see Soltis and Rieseberg, 1986). It has traditionally been assumed that autopolyploids are maladaptive and rare as a result of pairing difficulties from the formation of multivalents during meiosis. However, more recent studies have found that pairing control genes may result in the formation of bivalents in autopolyploids conferring allopolypoid-like behavior (Jackson, 1982; Soltis and Rieseberg, 1986;. Wolf et al. 1989). As a result, successful autopolyploids such as

Tolmiea menziesii, Medicago sativa, Medicago falcata, Coreopsis grandiflora var. *longipes* and *Heuchera grossulariifolia* are able to occur in natural populations.

In autopolyploids it is possible to have partial polysomy in which a portion of chromosomes are polyploid and others are diploid within the genome, as a result of gene silencing (Werth and Windham, 1991). Such individuals would display both disomic and polysomic inheritance. Such banding was seen with *M. macfarlanei*. Two of the dimeric enzymes, TPI and MDH had polysomic banding (see Fig. 3), whereas other enzymes such as PGI, ACON, SKDH, AAT and LAP appeared to have diploid banding (or were uninterpretable as polyploids) with some gene duplication. Since no known base number of chromosomes have been reported for *Mirabilis* we are unable to specify the precise level of ploidy, i.e. tetraploid, hexaploid, or octaploid. Without further investigation into the transmission genetics and extensive controlled crosses, it is difficult to determine the level of ploidy. We therefore interpreted the apparent polysomic enzymes as tetraploids, until further information is revealed.

The polysomic genotypes were used to calculate numbers of clones and genotypes per population, genetic distance and F_{ST} using allele frequencies. Diploid genotypes were needed in order to determine some measures of diversity such as: levels of heterozygosity, percentage of polymorphic loci, mean number of alleles per locus and fixation indices (F). Therefore for these analyses, the apparent polysomic genotypes were reduced to diploid genotypes (i.e. an AAAB or ABBB would be considered an AB).

Diversity

Genetic diversity--The various indices of genetic diversity for each population and the averages for all populations are presented in Table 4. The number of genotypes per population

ranged from as few as 7 genotypes at Cody Draw, to 43 at Long Gulch, with an average of 18.75 (SE 4.69) genotypes per population. This average is higher than the average number of genotypes/population (x=16.1, ranging from 1 to 167) found in the review of genetic diversity of 21 clonal plant species by Ellstrand and Roose (1987). Combining all *M. macfarlanei* populations from the Salmon there were 112 different genotypes. The number of genotypes per population increased as population size increased (Fig. 4). Lucile Caves was not included in this figure, since it was planted by the BLM and population size would not correlate with the number of genotypes. However, this population had 22 different genotypes in the two plots mapped, indicating a good diversity of rhizomes were planted.

Additional measures of genetic diversity are given in Table 4. On average 31.3% of the loci examined in each population were polymorphic (P), ranging from 12.5% at McKinzie Cr to 50.0% at Long Gulch. Percent polymorphic loci for each population are compared to population size estimates (number of stems/population) in Figure 5. The three smallest populations had higher P than the intermediate sized populations such as McKinzie and Lucus, although the two largest populations had the highest levels of polymorphic loci. The mean number of alleles per locus (A) averaged for all populations was 1.50, with a range from 1.3 and 1.8. Mean heterozygosity calculated as a direct count (H_{dc}) was 0.123 (SE 0.048) and Hardy-Weinberg expected heterozygosity (H_{exp}) was 0.129 (SE 0.037). In comparing all the diversity measures for populations, it appears that McKinzie Cr. was the least variable population, whereas Long Gulch was the most variable.

Genetic diversity values for *M. macfarlanei* were compared with other allozyme data for other plant species with similar life history, distribution and breeding systems reviewed in Hamrick

and God (1990) (Table 5). All of the categories selected for comparison reflect life history characteristics of MacFarlane's Four O'clock, i.e. long lived herbaceous perennial, endemic, mixed breeding systems. *Mirabilis macfarlanei* had lower percent polymorphic loci (31.3%) than the averages found in Hamrick and God's review paper (Table 5), including endemic species which had an average of 40% loci polymorphic. The average number of alleles per locus (1.5) was also lower than the compared averages. The expected mean heterozygosity (0.129) of *M. macfarlanei* was about average. It was higher than values for endemics and plants with mixed breeding systems, yet was in the same range as most dicot and plants with asexual and sexual reproduction. With the exception of the level of heterozygosity, values for genetic diversity of *M. macfarlanei* were lower than the averages for all of the categories examined.

Levels of Heterozygosity--Table 6 contains fixation indices (F) for all loci that were polymorphic in each population. Of the 47 polymorphic loci among all populations, 15 indicated excessive levels of heterozygotes (p), 17 were significantly deficient in heterozygotes and 15 were within the expected number of heterozygotes. No population was deficient or excessive for all loci. Instead, certain loci tended to be consistently excessive (or deficient) heterozygotes throughout all populations. For example, *Tpi-3* and *Mdh-3* consistently had excessive heterozygotes, while *Pgm-1, Act-1* and *Aat-1* were deficient in heterozygotes. Such a pattern of both excessive and deficient heterozygotes is unlikely to be indicative of inbreeding or outcrossing, but instead may be related to polyploidy and interpretation of loci.

Population differentiation

Nei's Genetic Distance--Nei's (1978) measure of unbiased genetic distance was used to estimate the genetic differences between pairs of populations. Values of genetic distance varied

between 0.004 and 0.141 with a mean of 0.056 (SE 0.007). These values are quite small indicating that populations share a lot of genetic information and are relatively undifferentiated from each other. Genetic distance values and geographic distance between pairs of populations are given in Table 7. The general genetic similarity among populations overcame any relationship between genetic differences and geographic distance between populations [2] (Fig. 6). Only a weak positive correlation between distance and genetic differentiation among populations was found (r = 0.084).

Measures of genetic distance were also used to construct a UPGMA dendrogram which displays the patterns of differentiation among populations (Fig. 7). From the dendrogram we see that Lucile Caves and John Day are very similar to each other, as would be expected since rhizomes planted at Lucile were taken from John Day. Two of the smallest populations: Cody Draw and Skookumchuck separate out from the other populations possibly as a result of a few fixed alleles in the small populations. Also it is noted that Lucus Draw is grouped among the seven populations south of it, rather than with its closest neighboring population, Cody Draw. Other groupings, also do not follow the expectation that closest populations are the most alike genetically.

Genetic variation within and among populations-- The amount of variation within and among populations was estimated using Wright's (1951) F-statistics. A summary of the F-statistics for all populations by locus is given in Table 8. A value of 0.059 for F_{IS} suggests little deviation in the number of heterozygotes from Hardy-Weinberg expectations. (Recall that an F_{IS}

[2] Pairs including Lucile Caves were not included in this analysis, as it was planted by the BLM and would not provide accurate information.

value of 0 suggests random mating and values close to 1 may indicate inbreeding. Negative values result from excessive heterozygosity.) Five of the loci, *Mdh-3, Mdh-2, Tpi-3, Tpi-1* and *Pgi-1,* have negative values indicating excessive heterozygosity over all populations, whereas four loci are positive indicating inbreeding. The *Skd-1* locus was the only one near zero. Therefore taking a mean over these 10 loci does not provide useful information.

The F_{ST} was relatively low (F_{ST}=0.206, with diploidized data; F_{ST} =0.263 with allele frequency data) indicating little population differentiation. F_{ST} was found to not be significantly different from zero (p=0.07) using a Chi-square test used by Workman and Niswander (1970).

The estimate of gene flow among the Salmon River populations was, *Nm* = 0.70 which is below the level needed to overcome genetic drift. *Aat-1* may account for this low measure of gene flow because of a fixed "d" allele in the Cody population. If this locus was excluded, the average F_{ST} would be 0.126 with a *Nm* of 1.73.

Clonal Structure

Population Maps--The clonal maps of Cody Draw, McKinzie Cr. and Skookumchuck populations are shown in Figs. 8, 9, 10 and 11. Colored circles indicate approximate size and location of stems in the populations. Circles represent one ramet, although multiple stems may branch above ground. The colors represent different genotypes from Cody, Skookumchuck and McKinzie and are given in Tables 9, 10 and 11 respectively. Cody Draw and Skookumchuck had 7 distinct genotypes in each population, while McKinzie Cr. had 8 genotypes. Clonal patches were delineated in Figs. 8 and 9 by outlining all ramets within 2.0m of each other with the same genotype. There were two reasons to select this 2.0m distance. First, Craig Johnson (BLM, personal communication) excavated rhizomes at John Day and found that internodes between

ramets were generally less than 5-6 feet (1.5m - 1.8m). Second, we found that like genotypes in the maps were generally closer than 2m. We should emphasize that these are only potential clones, determined by a limited number of loci. Stems with the same observed genotype may be different at other loci not examined, relegating them to different clones. In addition, the inter-ramet distance of a single clone may extend beyond the arbitrary 2.0m criterion. The number of clones per population was determined for each population. For Cody Draw and Skookumchuck the average number of ramets per clone was determined by counting the number of ramets within each clonal patch and averaging over all multi-ramet clones (this could not be determined for McKinzie Creek because not all stems were sampled).

There were an estimated 11 clones at Cody Draw (Fig. 8), two of which were individual stems. The number of ramets per multi-stemed clone ranged from 2 to 13 , with an average of 5.0 ramets per clone (SE 1.247). The average length of clones was 0.80m (SE 0.15), with the longest clone reaching 1.8 meters. In general, Cody Draw clones grew in fairly compact patches with some intermixing among clones. Some clumps where comprised of two or three different clones.

Seven distinct clones were identified at Skookumchuck (Fig. 9). Most of the population appears to be one clone (genotype 1, table 10), with a few small clones or individual stems (4 individual stems). The largest clone contained 34 ramets, while the remaining 6 clones had only 1 or 2 stems. Multi-ramet clones averaged 10 ramets per clone (SE 8) ranging from 2 to 34 ramets. The average length of clones was 0.94 m (SE 0.162), with the longest clone being 2.7m long.

Nine 3 ft x 3 ft plots are monitored by the BLM at Skookumchuck. In plot # 5, the BLM mapped stem placement and indicated stems as potential seedlings or mature *Mirabilis*. The clonal map of this plot as well as the corresponding BLM plot diagram is shown in figure 10.

Stems 7 and 9 from the clonal maps correspond to stems suggested to be seedlings in the BLM plot diagram. Both of these stems have nearby stems with identical genotypes, which suggests they are connected by a rhizome and perhaps not new seedlings. The majority of this population's diversity is located in this plot, especially when compared to the large clone covering most of the population. The several small genets or single stems located here are suggestive of seedling establishment during some point.

McKinzie Creek had 13 clones identified out of the 40 stems sampled (Fig. 11). Among the clumps sampled, there were frequently two or three different clones together. Because not all stems in a clump were sampled the number of ramets per clone would be underestimated and therefore was not calculated.

Long Gulch Plot -- In the 8 x 8 m plot at Long Gulch we observed 9 unique genotypes (Table 11) and identified 15 clones (Fig. 12). Seven of the fifteen genets were single stems, with no apparent vegetative spread during the current growing season. The other 8 multi-ramet clones ranged from 2 to 13 ramets with an average of 5 (SE 1.57) ramets per clone. The longest clone covered a distance of 6.09 m; the average clone length was 1.62 m (SE 0.73). Some clones were intermixed amongst each other, while others appeared to have a fairly dense distribution with many closely spaced ramets of the same genet.

Long Gulch Transect -- Eleven unique genotypes were determined from the stems sampled along the 5 ft x 435.5 ft belt transect #2. Stem location as measured along the transect and their genotypes are given in table 13. This profile of vegetation can show how long clones extend in a linear fashion. Like genotypes ranged from 0.075 m to 1.975 m with an average

length of 0.676 m (SE 0.287). Generally, stems along the transect were not intermixed with unlike genotypes.

Clump Samples -- Clumps of stems were sampled from Lucus Draw and McKinzie Creek to determine if "visual clones" (or clumps) were actually made up of one clone. At Lucus Draw, leaves were taken from two individual stems within each of 15 clumps. Of the 15 pairs of samples only 2 were unmatched in their genotypes indicating two different clones growing together (13% were made up of multiple clones). At McKinzie Cr., leaves from three individual stems were sampled within each of 11 clumps (6 stems were sampled from one large clump). Of the 11 patches, seven clumps contained unmatched genotypes, while only 4 had the same genotype for all three stems.

Lucile Caves Map -- In 1988 the BLM planted three sites at Lucile Caves with rhizomes from John Day. In site 1, rhizomes were planted in 30 scalp sites[3], while site 3 had 10 scalp sites. Sometimes multiple rhizomes were placed in one scalp site. This information was not recorded at the time, but can be inferred from genotype maps. Maps of stem genotypes in plots 1 and 3 were drawn to show where multiple rhizomes were placed in one scalp site (Fig. 13 and 14).

DISCUSSION

The genetic diversity of small, isolated populations may be low because of genetic drift, genetic bottlenecks, inbreeding, low gene flow or any combination of these factors.. MacFarlane's Four O'Clock, a rare endemic with small isolated populations in Idaho and Oregon, had lower

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[3] Rhizomes were planted in an area cleared of vegetation approximately 1.5 sq. ft. termed a "scalp site".

values of genetic diversity than many other plant species with similar life history traits. In particular, the number of polymorphic loci and number of alleles per locus were low. Because *M. macfarlanei* is polyploid, low diversity may have even greater significance, since most polyploids have higher numbers of alleles, polymorphic loci and levels of heterozygosity than do their diploid congeners (Soltis and Rieseberg, 1986; Wolf et. al. 1990).

Low levels of genetic diversity of *M. macfarlanei* populations are likely due, at least in part, to having experienced a genetic bottleneck. The common congeners of *M. macfarlanei* are widespread but mainly restricted to the warmer climates of the southwestern United States. Before the Pleistocene glaciation *M. macfarlanei* may have been more widespread, but with the advent of cooler climates the species or its predessor may have retreated to the warm pockets of Hell's Canyon and the Salmon River (Stebbins, 1979; Huenneke, 1991). Such historical reductions in distribution could have resulted in a genetic bottleneck. Genetic bottlenecks and random drift augmented by low gene flow among the remaining populations may have reduced the number of alleles per locus and thereby reduced the number of polymorphic loci.

The results of this study support the theory that small populations have reduced diversity. Overall, low levels of polymorphic loci and mean number of alleles per locus exist in *M. macfarlanei* populations. Yet, among populations sampled, the correlation between population size and the amount of genetic variation is weak. Although, the greatest levels of genetic diversity were found in the largest populations: John Day and Long Gulch, as expected, three of the smallest populations (Cody, Slicker Bar and Skookumchuck), maintained relatively high percent polymorphic loci (31.3% and 25% respectively), while intermediate sized populations (McKinzie Creek and Lucus Draw) had lower levels of polymorphic loci (see Fig. 5).

Lucus and McKinzie, the two populations with the lowest levels of polymorphic loci were monomorphic at several loci that were polymorphic in other populations. For instance, McKinzie Creek was monomorphic at three loci, *Tpi-3*, *Mdh-3* and *Skd-1* that were polymorphic in the other seven populations. Also a third "c" allele for *Lap-1* was not present at this population. Likewise, Lucus Draw was monomorphic at *Skd-1* and *Lap-1*, while the other 7 populations were polymorphic (except *Skd-1* for McKinzie Creek). These populations may have undergone a bottleneck and lost many of the alleles found throughout the other populations. Lucus Draw is one of the northernmost populations and is isolated from all other populations except Cody Draw. This may have isolated the population enough that little gene flow has occurred recently. McKinzie, on the other hand, is centrally located among the other populations. If gene flow was currently occurring among the populations we would expect McKinzie to have the same alleles as those found in other populations. Only a subset of all alleles and no unique alleles are found at McKinzie. This suggests that McKinzie may have undergone a population crash at sometime and lost those alleles. Without recruitment of new genetic material into populations, alleles may become fixed and polymorphisms may be lost through genetic drift.

Inbreeding reduces the proportion of heterozygotes in a population and thereby reduces genetic diversity. We expected selfing to be high in *M. macfarlanei* since it is self-compatible (Barnes, unpublished data) and displays extensive clonal growth. Handel (1985) suggested that with increased clonal density, ramets of self-compatible, entomophilous species experience more selfing because of intra-clonal pollinator movements. In some populations, such as Skookumchuck, the presence of widespread clones would be expected to increase the chance of selfing.

The data however did not support these predictions. The two parameters used to estimate levels of heterozygosity, the fixation index (F) and the inbreeding coefficient (F_{IS}), were not altogether indicative of inbreeding. The fixation index showed a mix of both excessive and deficient levels of heterozygotes for different loci (Table 6). Those loci that appeared to have polysomic inheritance were excessive in heterozygotes, i.e. *Tpi-3, Tpi-1, Mdh-3, Mdh-2*, and *Pgi-1* whereas other loci that were deficient in heterozygotes appeared to have disomic inheritance, i.e. *Aat-1, Skd-1* and *Lap-1*.

One explanation for excessive heterozygotes in *M. macfarlanei* is polyploidy. We expect greater heterozygosity in autopolyploids with polysomic inheritance than in diploids or disomic loci. Hardy-Weinberg assumes diploidy in which 2pq heterozygotes are expected from $2pq = 1-(p^2 + q^2)$. With an autopolyploid, there is an increased likelihood of heterozygosity since each locus is represented four times in tetraploids, six times in hexaploids and eight in octaploids. Selfing of an individual having *AABB* will result in a progeny ratio of 1*AAAA*: 8*AAAB*: 8*AABB*: 8*ABBB*: 1*BBBB* (Haldane, 1930; Soltis and Rieseberg, 1986). Therefore it is not surprising to see more heterozygotes in these loci than expected in a diploid populations. This is one reason why polyploid plants may be more adaptive than their diploid congeners.

If populations have gone through gene silencing, where the excessive copies of loci are no longer functional, some enzymes may be functioning as diploids (Werth and Windham, 1991). These disomic loci could therefore be indicative of levels of inbreeding occurring, and the fixation index and F_{IS} would be more meaningful. The data from the fixation index (Table 6) indicates that many of the populations are deficient in heterozygotes for *Skd-1* and *Lap-1*. In particular, Skookumchuck is deficient in heterozygotes for *Lap-1*. This may indicate high levels of

inbreeding have occurred in this very small population that is predominantly made up of one clone.

Some populations, such as Skookumchuck and Cody Draw, seemed to have fixed unbalanced heterozyogotes at *Tpi-3* and *Mdh-3* for all samples. Such a pattern where no homozygotes were observed is quite unusual and is probably a result of some type of heterozygote fixation. Alternatively, it may be that these genotypes were more successful and through natural selection more heterozygous individuals established than homozygous individuals. The unusual patterns of fixed heterozygotes and mix of polysomic and disomic inheritance throughout all of the populations warrants further investigation. We recommend that the inheritance of the isozymes be studied in order to understand the transmission genetics of *M. macfarlanei.*

Another measure of diversity is clonal distribution and numbers of genotypes per population. Of the three populations mapped, we see that Skookumchuck was markedly less diverse (Fig. 7, 8 and 10). Most ramets at Skookumchuck appear to belong to one clone, whereas Cody and McKinzie have fewer large clones and more small clones that were intermixed. All of the smaller populations (Cody, Skookumchuck, Slicker, McKinzie and Lucus) had significantly fewer genotypes than John Day and Long Gulch (Fig. 4). This is a function of the number of different clones in the populations and the amount of genetic diversity. When the Salmon populations of *Mirabilis macfarlanei* are compared to other clonal species, the average number of genotypes per population and total number of genotypes found throughout all populations is higher than most species in the Ellstrand and Roose (1990) review article. The source of such variation suggests that seedling establishment has occurred in the populations at

some time, even if little seedling establishment is occurring now (Ellstrand and Roose, 1987). Also, this indicates that populations are generally not composed of large expansive clones, but more discrete clones.

The amount of differentiation among populations can give an idea of current and past levels of gene flow. For the Salmon River populations Nei's genetic distance was 0.056, and F_{ST} was 0.256, indicating relatively little differentiation among most populations. Some unexpected results developed, such as the high level of differentiation between Lucus Draw and Cody Draw (D=0.11) which are separated by less than one kilometer. Cody Draw has a unique "D" allele at *Aat-1*, which is not found in most other populations. Also, Cody had three alleles at *Lap-1* and two alleles at *Skd-1*, whereas Lucus was monomorphic for both loci. The fact that none of these alleles were present at Lucus suggests that gene flow is not occurring between these populations or possibly that some type of reproductive barrier precludes crossing. We recommend controlled pollen crosses between the two populations to determine if viable seeds can be produced.

Wright's theory of "isolation by distance" predicts that with increased distance between populations lower levels of gene flow will occur and greater differentiation will occur among populations through genetic drift over time. Yet, the populations from the Salmon do not appear to have greater differentiation with increased distance (Fig. 6). Some genetic information is shared across all populations, suggesting that the separation among these populations has occurred fairly recently. At least recent enough that genetic drift has not caused significant differentiation among the populations. This should be contrasted with data from the USFS report (Barnes, Wolf and Tepedino, 1994) of the Snake and Imnaha River populations of *M. macfarlanei* in which a significant positive correlation was found among actual distance and

genetic distance among populations (r=0.92). This indicates that with increased distance populations were more differentiated. It is not surprising that the Snake and Imnaha populations are more differentiated, since they are more isolated from each other by geographic barriers, as well as from two different drainages. They have had sufficient time to have come into genetic equilibrium. Those populations from the Snake that were in close proximity of each other (West Creek/Kurry Creek complex) had D values similar to those found among the Salmon populations.

Many factors come into play for differentiation to occur among populations: levels of gene flow, time since separation and selective factors. Limited gene flow occurring among populations leads to greater population differentiation and lower genetic diversity through genetic drift. Gene flow can occur through pollination or seed dispersal. The predominant pollinators of *Mirabilis macfarlanei* are solitary bees: *Anthophora* spp. and *Bombus* spp. Old studies have found that *Anthophora* can fly up to 5 miles to return to their nesting sites when experimentally removed. However, it is unlikely that under normal pollen foraging flights such distances would occur. These visitors are polylectic (visitors of many species of plants) and are primarily visiting *Mirabilis* for nectar and not pollen, so would not depend strictly on *Mirabilis*. Therefore, it is unlikely that pollination is occurring among widely separated populations. It is not known how MacFarlane's Four O'Clock seeds are dispersed or how far they are moved and warrants further investigation. Because of their size and rugulous seed coat, they may be dispersed by mammals or birds. Regardless, seedling establishment appears to be infrequent (Craig Johnson, BLM and personal observation) and would limit the spread of genetic information (both pollen movement and seed dispersal) if seeds are not establishing. Furthermore, if gene flow was occurring among populations we would see a stronger association between populations that are very near each

other, i.e. Lucus Draw and Cody Draw, John Day and Long Gulch. Yet, some of these close populations are not closest genetically.

An alternative explanation for the low differentiation is that these populations were at one time more interconnected or one large panmictic population. In such a case, they would share genetic information. The amount of time since populations separated will effect population differentiation. If separation has occurred recently then populations will not be in equilibrium between genetic distances and geographic distances as genetic drift has not had time to take complete effect (Slatkin, 1993). This was seen with the Salmon populations, indicating that with time populations may become more differentiated.

Selection can also affect which genotypes are present in populations. If the plants are growing in populations which are relatively similar and are receiving the same selective forces, there may be little differences developing among the populations. There are similarities among the populations, including plant communities and soils. Likewise, weather patterns and rainfall levels are probably the same among the populations. If selective factors are similar, then populations would not be as likely to be differentiated, as seen in the Salmon populations.

In terms of conservation issues, several points are evident from this study. Overall, the populations had lower genetic diversity than plants reported in the review article by Hamrick and Godt (1990). A comparison of genetic diversity of *Mirabilis macfarlanei* with its close congeners would provide more information and may reveal information about polyploidy in the genus. It is hard to say how levels of diversity have changed over time for populations of *M. macfarlanei*, but occasional monitoring of genetic variation can inform managers if genetic erosion is occurring within populations (Ellstrand and Elam, 1993).

Among the populations surveyed, the larger populations tended to have greater genetic diversity, yet some of the smaller populations had a considerable amount of diversity, which stresses the need to protect even small populations. McKinzie Cr. appeared the least genetically diverse, while Long Gulch and John Day maintained the most genetic diversity. Because Long Gulch and John Day were the most diverse and shared many alleles with other populations, they may be best suited for use in reintroduction projects such as the one at Lucile Caves.

The clonal maps revealed differences in clonal growth forms among the populations, which makes it difficult to give a general description of clonal size for the species. At McKinzie Creek what visually appeared to be a clone, based on a clump of stems often times had several clones growing together. However, Lucus Draw appeared to have clumps that were of one clone. From all populations mapped it was evident that the number of ramets does not reflect the number of individuals in the population. For example, of the 49 stems present at Cody Draw only 13 clones were identified. Likewise, Skookumchuck had only 8 clones out of 50 stems present. This should be considered when estimating population sizes.

Glossary

Allele - One of two or more forms that can exist at a single gene locus, distinguished by their differing effects on the phenotype. In a diploid, two alleles are possible at a particular locus. For example, two different forms of the same enzyme can occur in an individual.

Bottleneck - A sharp reduction in the number of individuals of a species in a particular time or place. A genetic bottleneck causes a loss of genetic diversity as a result of genetic information is lost from lost individuals.

Clone - A genetically uniform assemblage of individuals (ramets) derived from a single genetic individual (genet). Vegetative growth through stolons, rhizomes, corms, bubils, root buds, etc.

Disomic inheritance - Genetic information which is inherited like a diploid. Each locus will have two alleles passed on from two chromosomes.

Gamet - A haploid sex cell.

Genet - Collective name for all ramets of the same individual.

Genome - One complete set of genetic information from a genetic system.

Genetic drift - random loss and fixation of alleles, due to variations in genetic information passed on from generation to generation.

Heterozygote - An individual or locus that maintains two different alleles for a specific locus.

Homozygote - An individual or locus that maintains two copies of the same allele at specific locus.

Locus - An area on a chromosome which codes for some type of a function in the organism, i.e. an enzyme is made by a particular locus on a chromosome.

Polysomic inheritance- Inheritance of an autopolyploid, in which there are two or more alleles per locus depending on the ploidy level i.e. a tetraploid will have four alleles, a hexaploid will have six alleles. Each chromomosome present with that locus will provide an allele.

Ramet - Vegetative offspring of a clonal plant.

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TABLE 1. Location of selected populations of *Mirabilis macfarlanei* used for study and sample size

Population	Element Occurrence[a]	Approx. Size[b]	No. of samples[c]
Cody Draw (Giants Nose 1)	0005	50-150	49 (12)
John Day	0002	3,000	42 (42)
Lucus Draw (Giants Nose 2)	0005	500-1,000	40 (24)
Long Gulch	0013	7,000	40 (40)
Lucile Caves	0008	180	35 (29)
McKinzie Creek		300	38 (14)
Slicker Bar	0004	150	40 (40)
Skookumchuck	0001	50-100	38 (8)

[a] Element occurrence numbers from Natural Heritage Program identification numbers.

[b] Size approximation is given as number of stems in the population taken from BLM reports and data.

[c] Number of samples collected are listed. The number of samples used in the population analysis in parentheses.

TABLE 2. List of all enzyme and buffer systems tried in preliminary work. Buffer system numbers refer to recipes used by Soltis et al. (1983); morpholine-citrate (MC) refers to buffers used by Odrzykoski et al. (1984). Resolvability of banding, number of loci visible and polymorphisms detected are indicated

Enzymes		EC Numbers	Buffer Systems	Resolv-able	# Loci	Poly-morphic
AAT	Aspartate aminotransferase	2.6.1.1	MC, 8, 6	yes	2	yes
ACPH	Acid phosphatase	3.1.3.2	6, 8, 1, 11	no	2	no
ACON	Aconitase	4.2.1.3	MC, 1, 11, 8	yes	3	yes
ADH	Alcohol dehydrogenase		1, 11, 6, 8	no	-	no
ALD	Aldolase	4.1.2.13	MC, 11	no	1	no
F16D	Fructose 1,6 dehydrogenase	3.1.3.11	MC, 1, 9, 11	no	2	yes
GDH	Glutamate dehydrogenase	1.4.1.2	1, 11	no	-	no
G3PD	Glycerol-3-phosphate dehyd.	1.2.1.12	MC, 11	no	-	no
G6PD	Glucose-6-phosphate dehyd.	1.1.1.49	8, 11	no	-	no
HK	Hexokinase	2.7.1.1	6, 8, 9, 11	no	2	no
IDH	Isocitrate dehydrogenase	1.1.1.42	MC, 11	yes	1	no
LAP	Leucine aminopeptidase	3.4.11.-	MC, 6, 8	yes	1	yes

TABLE 2. CONT.

Enzymes		EC Numbers	Buffer Systems	Resolv-able	# Loci	Poly-morphic
MDH	Malate dehydrogenase	1.1.1.37	MC, 1, 9, 11	yes	3	yes
MNR	Melnadione reductase		1, 11	no	-	no
6PGD	6-phospho-gluconate dehyd.	1.1.1.44	MC	no	6	no
PGI	Phosphoglucose isomerase	5.3.1.9	MC, 6, 8	yes	3	yes
PGM	Phospho-glucomutase	2.7.5.1	1, 6, 8, 11	yes	3	yes
SKDH	Shikimate dehydrogenase	1.1.1.25	MC, 1, 11, 9	yes	1	yes
SOD	Superoxidase dimutase		8	no	1	yes
TPI	Triose-phosphate isomerase	5.3.1.1	MC, 6, 8	yes	3	yes

TABLE 3. Allele frequencies by locus for all populations. Populations 1 through 8 are as follows: Cody Draw, John Day, Lucus Draw, Long Gulch, Lucile Caves, McKinzie Creek, Skookumchuck and Slicker Bar

Locus	Allele	1	2	3	4	5	6	7	8
Tpi-3	A	0.71	0.56	0.44	0.76	0.57	1.00	0.79	0.80
	B	0.29	0.44	0.56	0.24	0.43	0.00	0.21	0.20
Tpi-2	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Tpi-1	A	1.00	1.00	1.00	1.00	0.98	1.00	0.93	1.00
	B	0.00	0.00	0.00	0.00	0.02	0.00	0.07	0.00
Pgm-1	A	0.92	0.95	1.00	0.90	0.89	1.00	1.00	0.97
	B	0.08	0.05	0.00	0.08	0.04	0.00	0.00	0.00
	C	0.00	0.00	0.00	0.02	0.07	0.00	0.00	0.03
Pgm-2	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mdh-3	A	0.79	0.83	0.77	0.51	0.98	1.00	0.71	0.18
	B	0.21	0.09	0.21	0.10	0.02	0.00	0.29	0.32
	C	0.00	0.08	0.04	0.39	0.00	0.00	0.00	0.48
Mdh-2	A	1.00	1.00	1.00	0.89	1.00	1.00	1.00	1.00
	B	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00
Mdh-1	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Skd-1	A	0.04	0.11	0.00	0.30	0.07	0.04	0.00	0.15
	B	0.96	0.89	1.00	0.70	0.93	0.96	1.00	0.85
Idh-1	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lap-1	A	0.17	0.05	0.00	0.05	0.13	0.11	0.31	0.10
	B	0.50	0.86	1.00	0.85	0.83	0.89	0.31	0.68
	C	0.33	0.09	0.00	0.10	0.04	0.00	0.38	0.22

TABLE 3. CONT.

Locus	Allele	1	2	3	4	5	6	7	8
Acn-1	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Acn-2	A	0.91	0.33	0.47	0.62	0.19	0.37	0.00	0.78
	B	0.00	0.48	0.05	0.00	0.69	0.08	1.00	0.00
	C	0.09	0.19	0.48	0.38	0.12	0.37	0.00	0.21
	D	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00
Acn-3	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pgi-1	A	0.00	0.22	0.00	0.00	0.08	0.04	0.00	0.00
	B	1.00	0.78	1.00	1.00	0.92	0.96	1.00	1.00
Aat-1	A	0.00	1.00	1.00	0.92	0.88	1.00	1.00	1.00
	B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	1.00	0.00	0.00	0.08	0.12	0.00	0.00	0.00

TABLE 4. Measures of genetic diversity within populations of *Mirabilis macfarlanei* (standard error in parantheses)

Population	No. of genotypes per population	Mean no. of alleles per locus	Percentage of loci poly-morphic	Mean Heterozygosity	
				Direct count	HdyWbg exp.
Cody Draw	7	1.4	31.3	.156	.131
John Day	34	1.6	37.5	.133	.154
Lucus Draw	13	1.3	18.8	.103	.100
Long Gulch	43	1.7	50.0	.167	.189
Lucile Caves	22	1.8	43.8	.096	.131
McKinzie Cr	8	1.4	12.5	.023	.063
Skookumchuck	8	1.3	25.0	.157	.121
Slicker Bar	15	1.5	31.3	.150	.141
Mean	18.75 (4.72)	1.5 (.185)	31.3 (4.41)	.123 (.048)	.129 (.037)

TABLE 5. Average levels of genetic diversity for plants with life history traits matching *Mirabilis macfarlanei*. Taken from Hamrick and Godt, 1990.

Categories	P	A	H_{exp}
MIMA	31.3	1.50	0.129
Dicot	44.8	1.79	0.136
Herb. Perennial	39.6	1.42	0.205
Endemic	40.0	1.80	0.096
Temperate	48.5	1.91	0.146
Mixed-Animal breeding	40.0	1.68	0.120
Seed gravity	45.7	1.81	0.136
Sexual/asexual	43.8	1.69	0.138

TABLE 6. Coefficient for heterozygote deficiency or excess, as determined by the fixation index (F) where $F = [1 - (Het_{obs}/Het_{exp})]$. F < zero indicates excessive heterozygotes and F > zero indicates deficient heterozygotes[1]. Only those locus which were polymorphic at each population were analyzed, none polymorphic locus indicated by ****.

	Populations							
Locus	Cody Draw	John Day	Lucus Draw	Long Gulch	Lucile Caves	McKinzie Creek	Skook-umchk	Slicke Bar
Tpi-3	-1.00**	-0.82**	-0.46*	-0.44**	-0.79**	****	-1.00**	-0.63**
Tpi-1	****	****	****	****	-0.02	****	-0.08	****
Pgm-1	-0.09	0.65**	****	0.15	0.64**	****	****	1.00**
Mdh-3	-0.85**	-0.25*	-0.74**	-0.34**	-0.04	****	-1.00**	-0.59**
Mdh-2	*****	****	****	-0.11	****	****	****	****
Skd-1	-0.04*	0.13	****	-0.31*	-0.08	-0.04	****	0.61**
Lap-1	0.45*	0.69**	****	0.41**	0.42*	-0.12	0.44	-0.08
Acn-2	1.00**	1.00**	1.00**	1.00**	1.00**	1.00**	****	****
Aat-1	****	****	****	1.00**	1.00**	****	****	1.00**
Pgi-1	****	-0.17	****	****	-0.09	-0.04	****	****

[1]Significance by Chi-square analysis indicated by * for $p<0.05$ and ** $p<0.01$.

TABLE 7. Nei's (1978) measure of unbiased genetic distance and geographic distance between all pairs of the eight populations are given.

Populations	Genetic Distance	Distance (km)
Cody/John Day	0.11	19.8
Cody/Lucus	0.1	0.4
Cody/Long Gulch	0.09	17.5
Cody/Lucile Caves	0.1	
Cody/McKinzie Cr.	0.1	12.2
Cody/Skookumchuck	0.14	6.2
Cody/Slicker Bar	0.09	15.2
John Day/Lucus Draw	0.02	19.4
John Day/Long Gulch	0.03	1.8
John Day/Lucile	0.004	
John Day/McKinzie	0.02	7.9
John Day/Skookumchuck	0.03	13.7
John Day/Slicker Bar	0.05	4.6
Lucus/Long Gulch	0.02	17.1
Lucus/Lucile	0.03	14.8
Lucus/McKinzie	0.02	11.8
Lucus/Skookumchuck	0.08	5.8
Lucus/Slicker Bar	0.04	14.8

TABLE 7. CONT.

Populations	Genetic Distance	Distance (km)
Long Gulch/Lucile	0.05	
Long Gulch/McKinzie	0.02	0.7
Long Gulch/Skookumchuck	0.09	5.5
Long Gulch/Slicker	0.01	2.5
Lucile/McKinzie	0.03	
Lucile/Skookumchuck	0.03	
Lucile/Slicker Bar	0.07	
McKinzie/Skookumchuck	0.06	6.2
McKinzie/Slicker Bar	0.05	3.4
Skookumchuck/Slicker Bar	0.08	9.1

TABLE 8. Summary of F-statistics for all loci in all populations. F_{ST} was calculated using Wright 1978 with allele frequency data (polyploid genotypes)

Locus	F_{IS}	F_{ST}	F_{IT}
Tpi-3	-0.677	0.102	-0.538
Tpi-1	-0.046	0.000	-0.005
Pgm-1	0.411	0.021	0.429
Mdh-3	-0.497	0.229	-0.173
Mdh-2	-0.114	0.080	-0.022
Skdh-1	0.033	0.092	0.121
Lap-1	0.308	0.152	0.414
Acn-2	1.000	0.351	1.000
Aat-1	1.000	0.824	1.000
Pgi-1	-0.151	0.110	0.027
Mean	0.059	0.263	0.253

Table 9. Genotypes for Cody Draw population for clonal map shown in Fig.8. Only polymorphic are given.

Genotype	Color	*Tpi-3*	*Mdh-3*	*Pgm-1*	*Skd-1*	*Lap-1*	*Acn-1*	*Aat-1*
1		AAAB	AAAB	AA	BB	BB	AA	DD
2		AAAB	AAAB	AA	BB	AC	AA	DD
3		AAAB	AAAB	AA	BB	CC	AA	DD
4		AAAB	AAAA	AA	BB	AC	AA	DD
5		AAAB	AAAB	AB	AB	AC	AA	DD
6		AAAB	AAAB	BB	BB	CC	CC	DD
7		AAAB	AAAB	BB	BB	BB	AA	DD

Table 10. Genotypes for Skookumchuck population clonal map shown in Figs.9 and 10. Only polymorphic loci are given.

Genotype	Color	*Tpi-3*	*Tpi-1*	*Mdh-3*	*Skd-1*	*Lap-1*	*Acn-2*
1		AAAB	AAAA	AAAB	BB	BB	BB
2		AAAB	AAAA	AAAB	BB	AB	BB
3		AAAB	AAAA	AAAB	BB	CC	BB
4		AAAB	AAAA	AAAB	BB	AA	BB
5		AAAB	AAAA	AAAB	BB	AC	BB
6		AAAB	AAAA	AAAB	BB	AC	BB
7		AAAB	AAAA	AAAB	BB	AA	BB

Table 11. Genotypes for McKinzie Creek population for clonal map shown in Fig.11 Only polymorphic given

Genotype	Color	*Tpi-3*	*Mdh-3*	*Pgm-1*	*Skd-1*	*Skd-1*	*Acn-1*	*Pgi-1*
1		AAAA	AAAA	AA	BB	BB	CC	BB
2		AAAB	AAAB	AB	AB	AB	DD	AB
3		AAAA	AAAA	AD	BB	BB	CC	BB
4		AAAB	AAAA	AA	BB	BB	AA	BB
5		AAAA	AAAA	AB	BB	BB	AA	BB
6		AAAA	AAAA	AA	AB	AB	CC	BB
7		AAAA	AAAA	AA	AB	AB	CC	BB
8		AAAA	AAAA	AA	BB	BB	BB	BB

Table 12. Genotypes for Long Gulch 8m x 8m plot for clonal map shown in Fig. 12. Only polymorohic loci are given

Genotype	Color	*Tpi-3*	*Mdh-3*	*Skd-1*	*Lap-1*	*Acn-2*
1		AAAABB	AABBCC	BB	AA	BB
2		AAABB	AABBCC	BB	BC	AA
3		AAAABB	AAACCC	BB	CC	BB
4		AAAB	AAAA BB	BB	BB	AA
5		AAAABB	AAABBC	BB	BB	AA
6		AAAABB	AAABBC	BB	AB	AA
7		AAAAB	AAABBC	BB	AA	AA
8		AAAABB	AAABBC	BB	BC	AA
9		AAAABB	AAABBC	BB	CC	AA

TABLE 13. Genotypes for Long Gulch Transect # 2 (5ft x 435.5ft belt transect) for clonal map. Each number indicates a unique genotype. Linear distances from the zero start point are given in feet and meters. If two or more stems were located at one point, all stems were sampled and labeled "a", "b" etc. Only polymorphic loci are given

Geno type	Linear Distance (Ft)	Linear Distance (M)	*Tpi-3*	*Mdh-3*	*Mdh-2*	*Skd-1*	*Lap-1*	*Acn-2*
1	4'5"	1.32	AAAB	ABBC	AABB	AB	BB	CC
1	5'5"	1.62	AAAB	ABBC	AABB	AB	BB	CC
1	6'3"	1.87	AAAB	ABBC	AABB	AB	BB	CC
1	7'4"	2.20	AAAB	ABBC	AABB	AB	BB	CC
1	11'	3.30	AAAB	ABBC	AABB	AB	BB	CC
2	30'	9.00	AABB	AAAA	AAAA	BB	BC	AA
3	30'9"	9.22	AAAB	AABC	AABB	AA	BC	AA
3	31'	9.30	AAAB	AABC	AABB	AA	BC	AA
4	135'	16.20	AAAA	BBCC	AAAA	BB	AB	BB
4	135'2"a	16.22	AAAA	BBCC	AAAA	BB	AB	BB
4	135'2"b	16.22	AAAA	BBCC	AAAA	BB	AB	BB
4	137'	16.44	AAAA	BBCC	AAAA	BB	AB	BB
5	176'	21.12	AAAA	AACC	AABB	BB	CC	
5	178'	21.36	AAAA	AACC	AABB	BB	CC	
6	254'5"	76.32	AAAA	AACC	AABB	BB	AA	AA
6	255' a	76.50	AAAA	AACC	AABB	BB	AA	AA
6	255' b	76.50	AAAA	AACC	AABB	BB	AA	AA
6	255' c	76.50	AAAA	AACC	AABB	BB	AA	AA
7	256'1"	76.83	AAAA	ABBC	AABB	BB	BB	
9	273'7"a	82.07	AAAB	ABBC	AABB	AA	AA	AA
9	273'7" b	82.07	AAAA	ABBC	AABB	AA	AA	AA
9	274'3"	82.27	AAAA	ABBC	AABB	AA	AA	AA

TABLE 13. CONT.

Geno type	Linear Distance (Ft)	Linear Distance (M)	*Tpi-3*	*Mdh-3*	*Mdh-2*	*Skd-1*	*Lap-1*	*Acn-2*
9	274'4"	82.30	AAAA	ABBC	AABB	AA	AA	AA
9	275'2"	82.55	AAAA	ABBC	AABB	AA	AA	AA
	275'4"	82.60	AAAA			AA	AA	AA
9	275'6"	82.65	AAAA	ABBC	AABB	AA	AA	AA
9	278'9"	83.62	AAAA	ABBC	AABB	AA	AA	AA
9	279'5"	83.82	AAAA	ABBC	AABB	AA	AA	AA
9	280'1"	84.02	AAAA	ABBC	AABB	AA	AA	AA
10	286'9"	86.02	AAAA	ABCC	AAAA	AB	CC	AA
11	287'1"	86.35	AAAB	ABCC	AAAA	AB	CC	AA
11	287'9"	86.32	AAAB	ABCC	AAAA	AB	CC	AA
10	288'1"	86.42	AAAA	ABCC	AAAA	AB	CC	AA
11	288'3"	86.47	AAAB	ABCC	AAAA	AB	CC	AA

FIG. 1. Approximate area of *Mirabilis macfarlanei* populations along the Salmon River and Snake River.

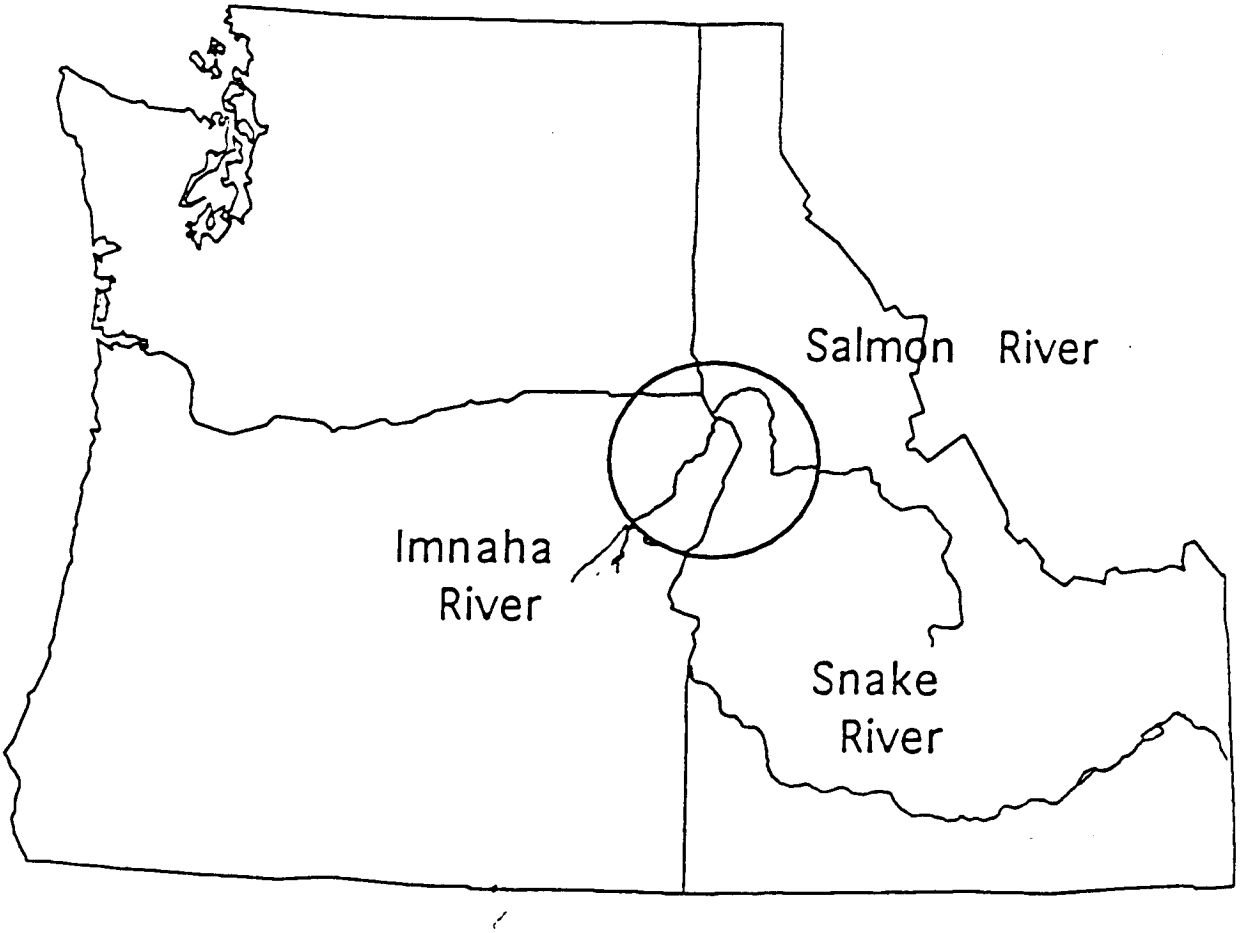


FIG. 2 Simplified flow chart of enzyme electrophoresis methodology.

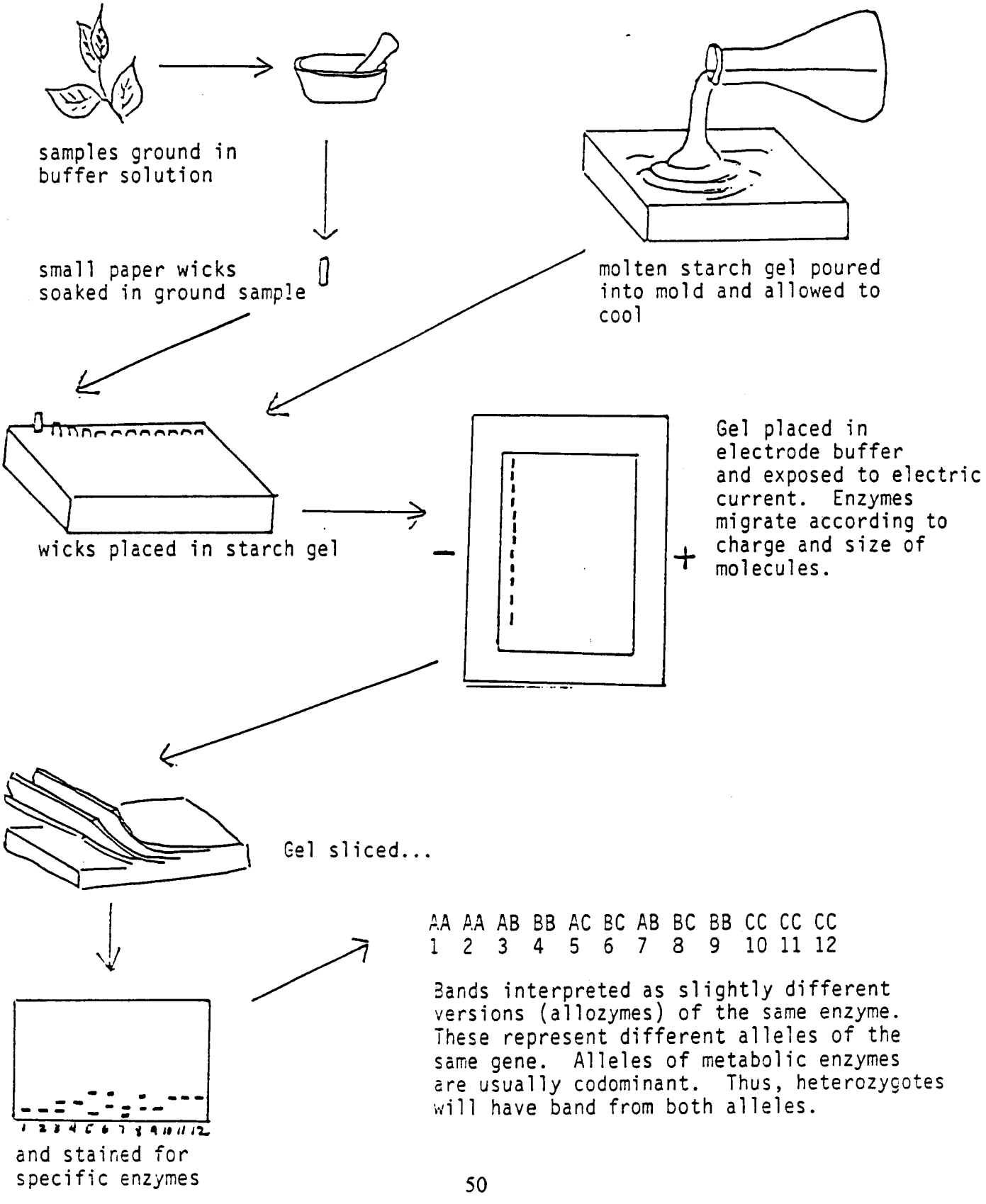


FIG. 3. Photographs of starch gels stained for SKDH, LAP, and TPI showing polymorphisms for *M. macfarlanei*. 3a. Two alleles shown for the *Skdh-1* locus. 3b. Three alleles shown for *Lap-1*. 3c. Three loci shown for TPI. The top locus, *Tpi-3*, is polymorphic showing tetraploid banding of a dimeric enzyme: (samples 1-14, AAAA; samples 17, 19, 21-23, AAAB; sample 18 AABB; samples 15, 16, 20, 25, 26, 28 ABBB; sample 24 BBBB; sample 29 CCCC).

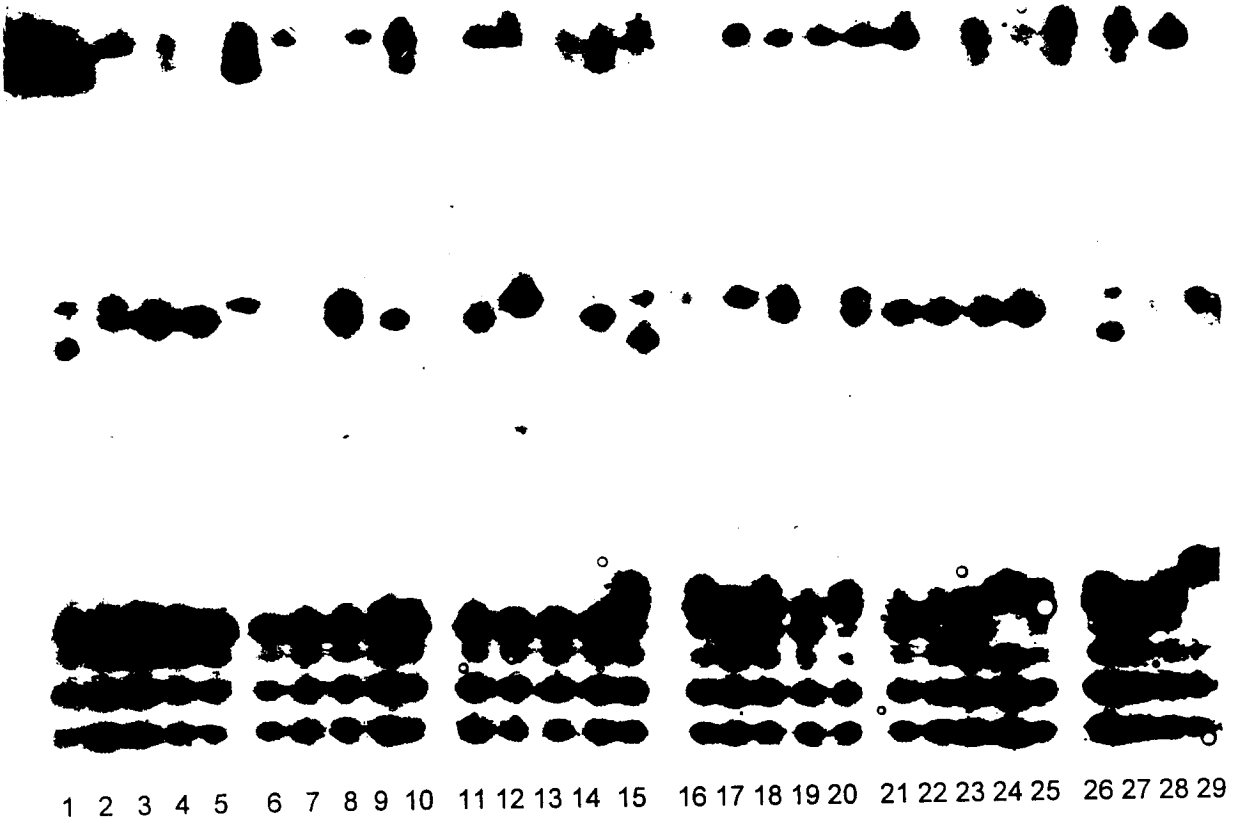


FIG. 4. Comparison of the number of genotypes found in populations from 40+ samples to the estimated number of stems in populations (BLM information)

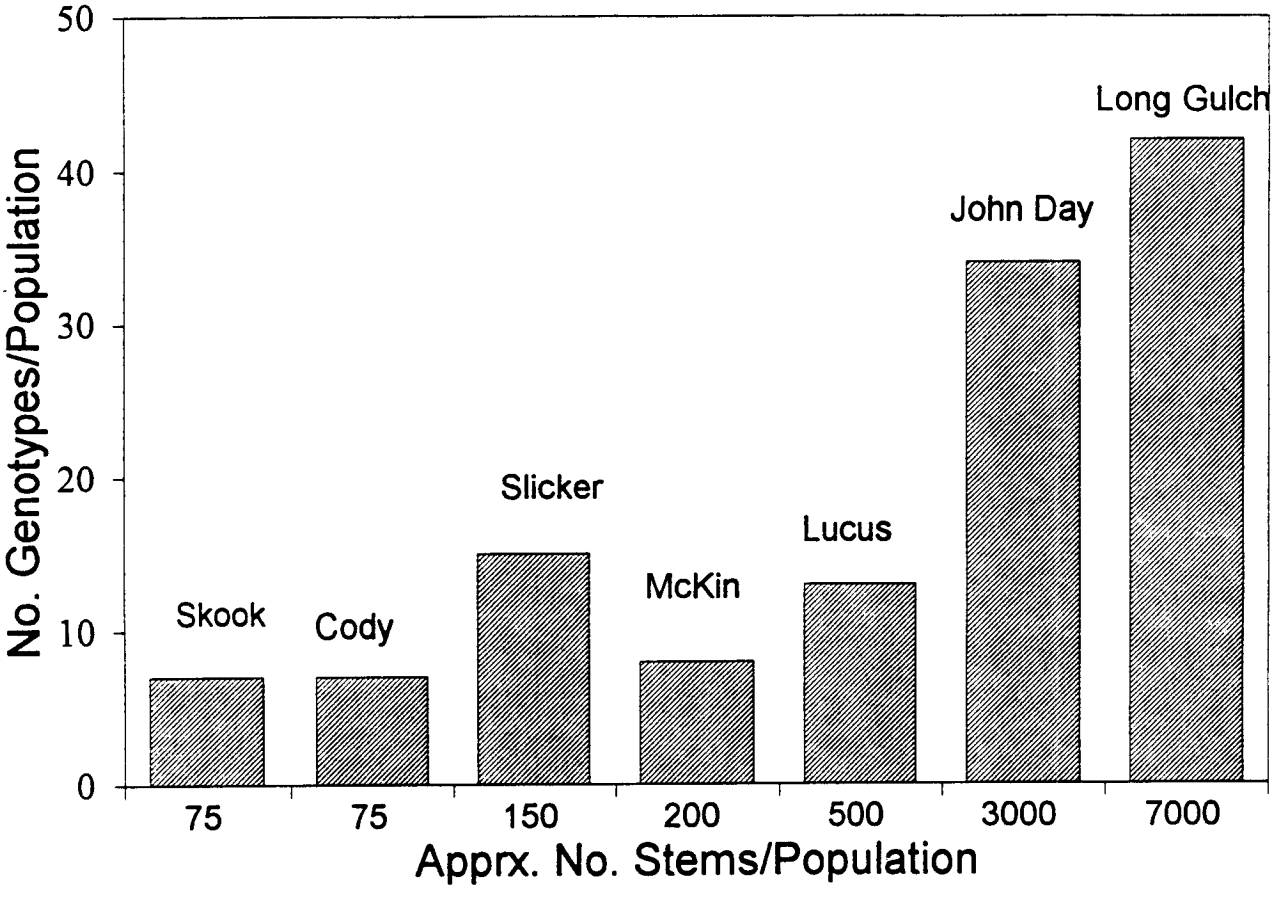


FIG. 5. Comparison of the percent polymorphic loci in each population to the estimated number of stems in populations

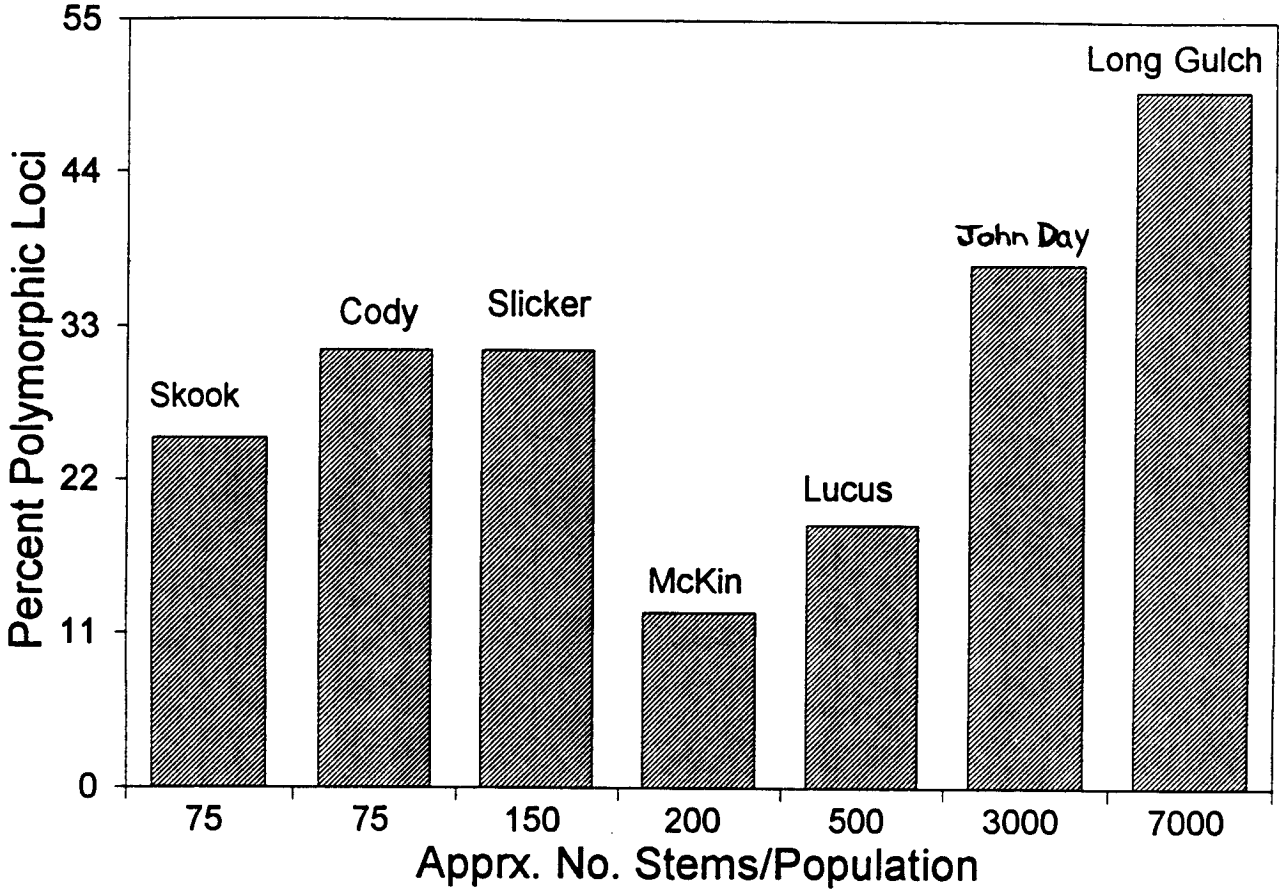


FIG. 6. Comparison of Nei's (1978) unbiased genetic distance (GD) versus geographical distance (Dist) between Salmon River populations. Resulting correlation coefficient (r) was 0.084.

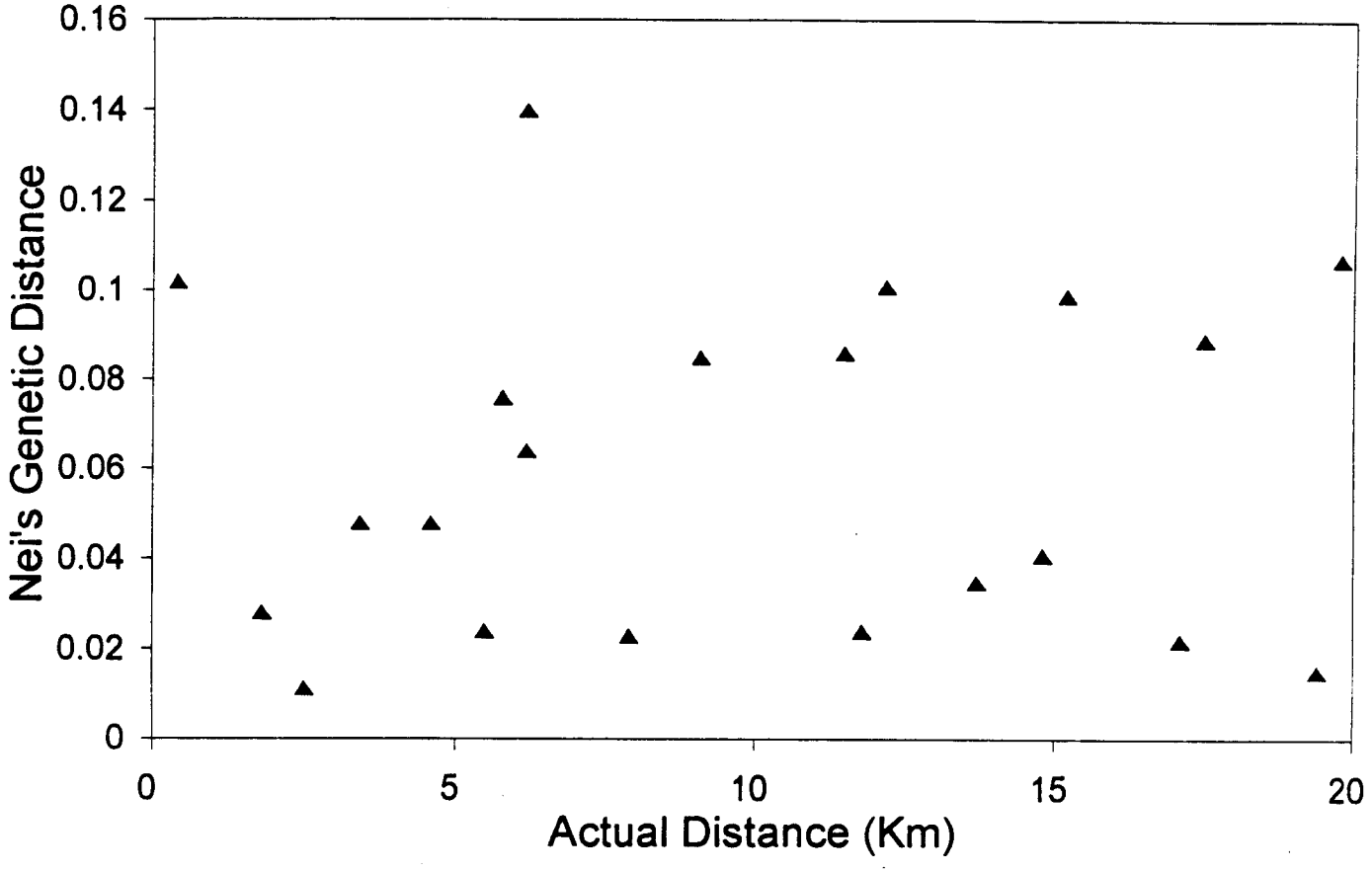


FIG. 7. The results of a UPGMA cluster analysis using Nei's (1978) unbiased genetic distance.

```
                                 Distance

.20       .17       .13       .10       .07       .03       .00
+----+----+----+----+----+----+----+----+----+----+----+----+

                             ********************************  CODY DRAW
                             *
                             *                              **  JOHN DAY
                             *                         ******
                             *                       ***    **  LUCILE CAVES
                             *                       * *
                             *                   ***** *******  LUCUS DRAW
                             *                   *   *
                             *            ********   ********* MCKINZIE CR
                             *            *      *
                             *            *      *        ****  LONG GULCH
                             **************      **********
                                          *               ****  SLICKER BAR
                                          *
                                          ******************** SKOOKUMCHUK

+----+----+----+----+----+----+----+----+----+----+----+----+
.20       .17       .13       .10       .07       .03       .00
```

FIG.8. Clonal map of Cody Draw population. Different patterns indicate the 7 unique genotypes given in Table 9. Each circle represents a single ramet, approximating plant size and location in the population. Unpatterned ramets are unknown genotypes, due to leaf material. Outlined ramets with cross hatches indicate estimated clonal patches.

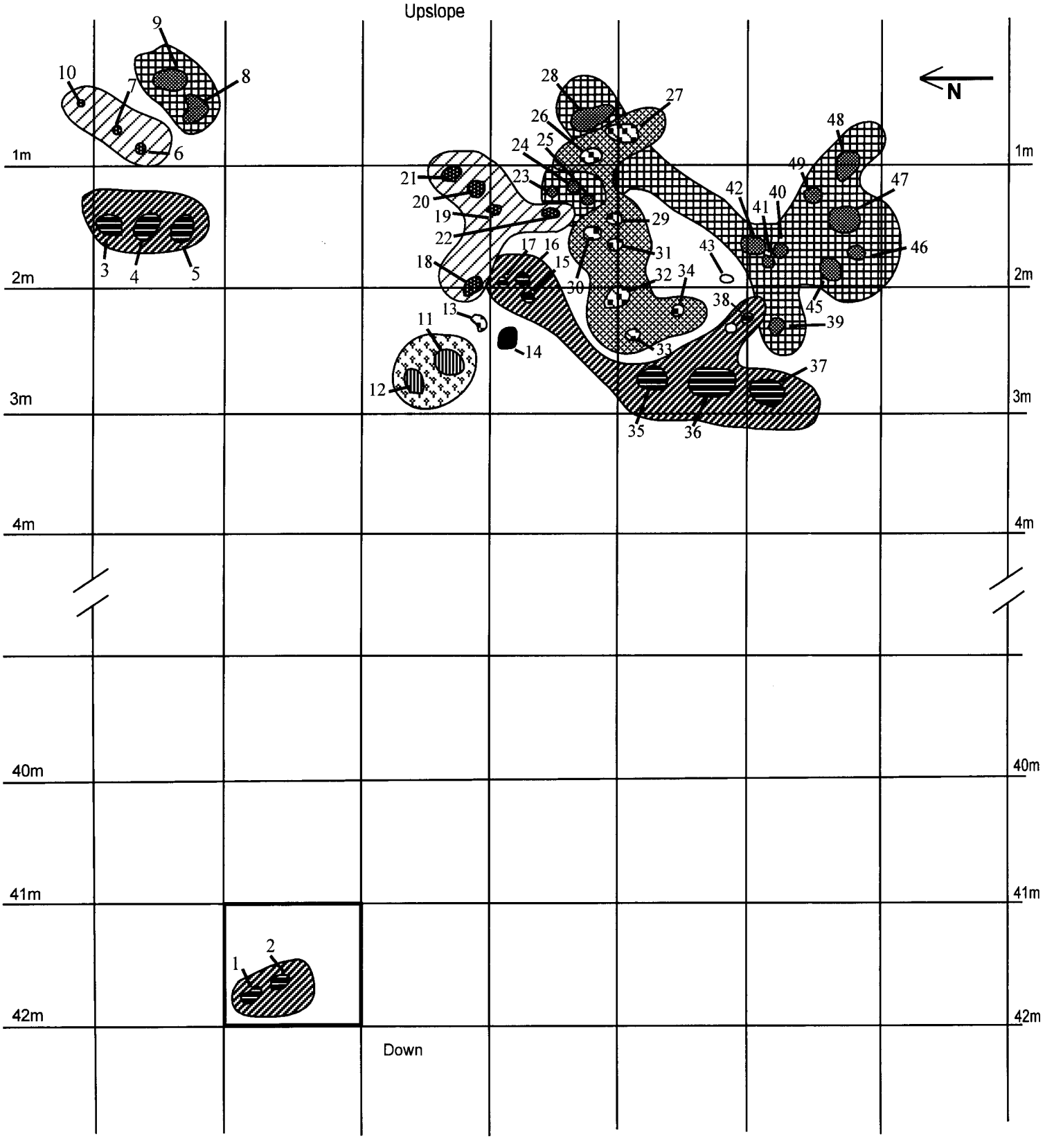


FIG. 9. Clonal Map of Skookumchuck population. Different patterns indicate the 7 unique genotypes given in table 10. Each circle represents a single ramet, approximating plant size and location in the population. Unpatterned ramets are unknown genotypes, due to bad leaf material. Outlined ramets indicate estimated clonal patches.

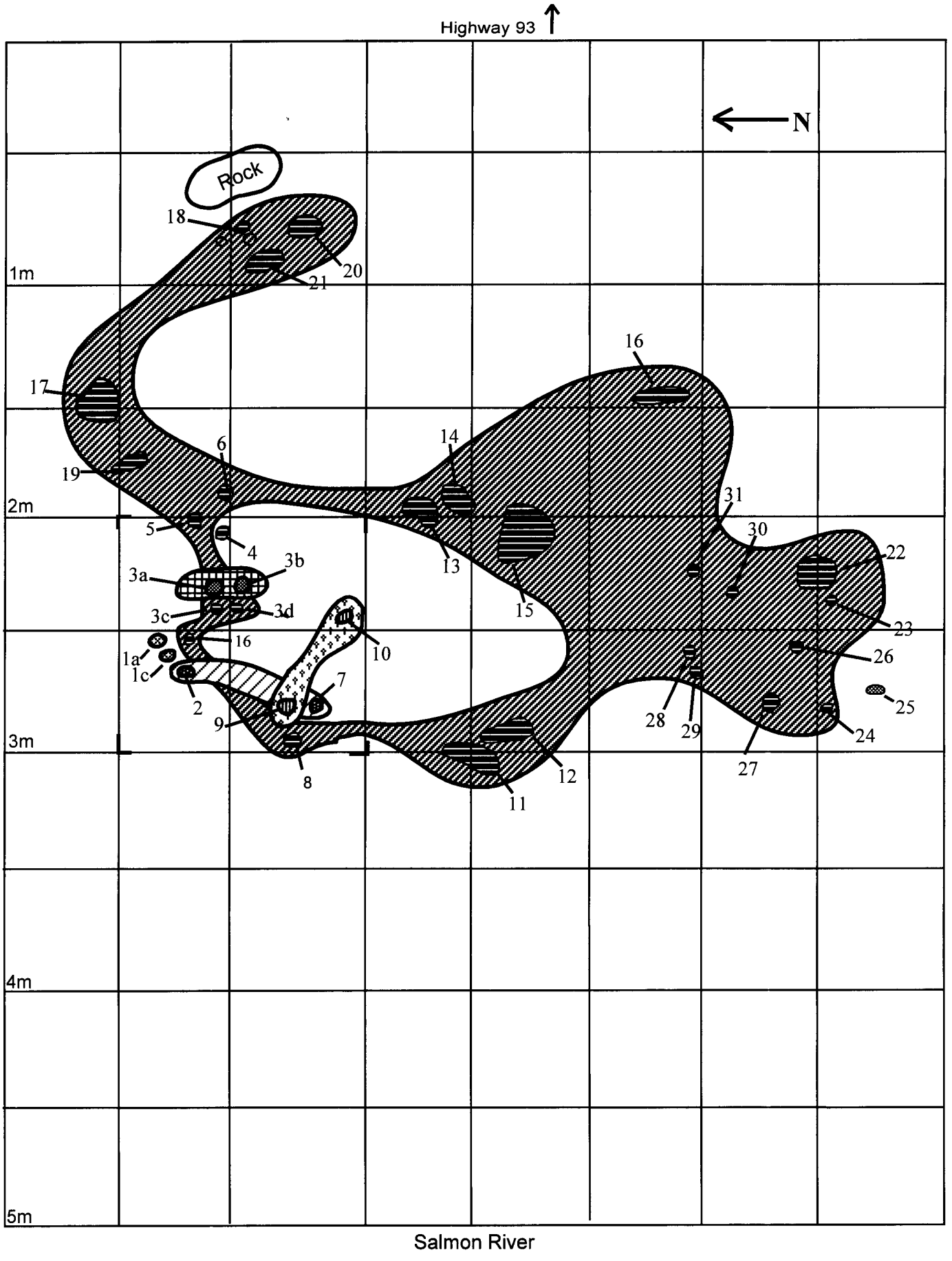
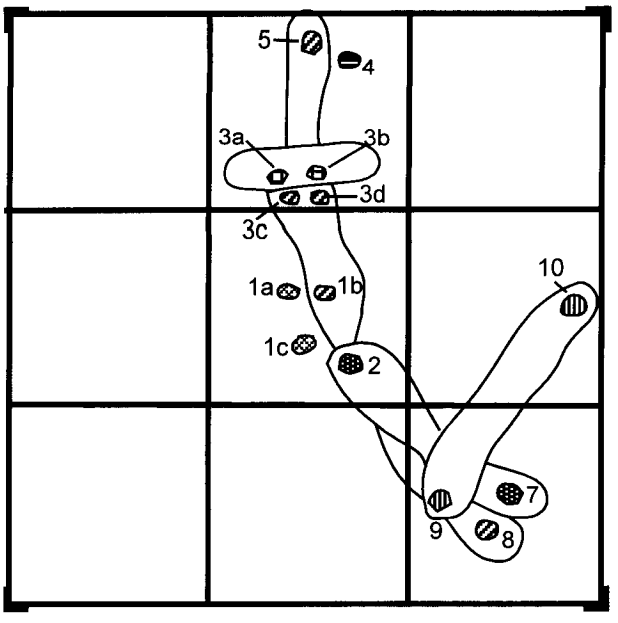


FIG.10. Clonal map and monitouring plot of BLM3ftx3ft plot #5 from Skookumchuck.



PART III-PLOT DIAGRAM

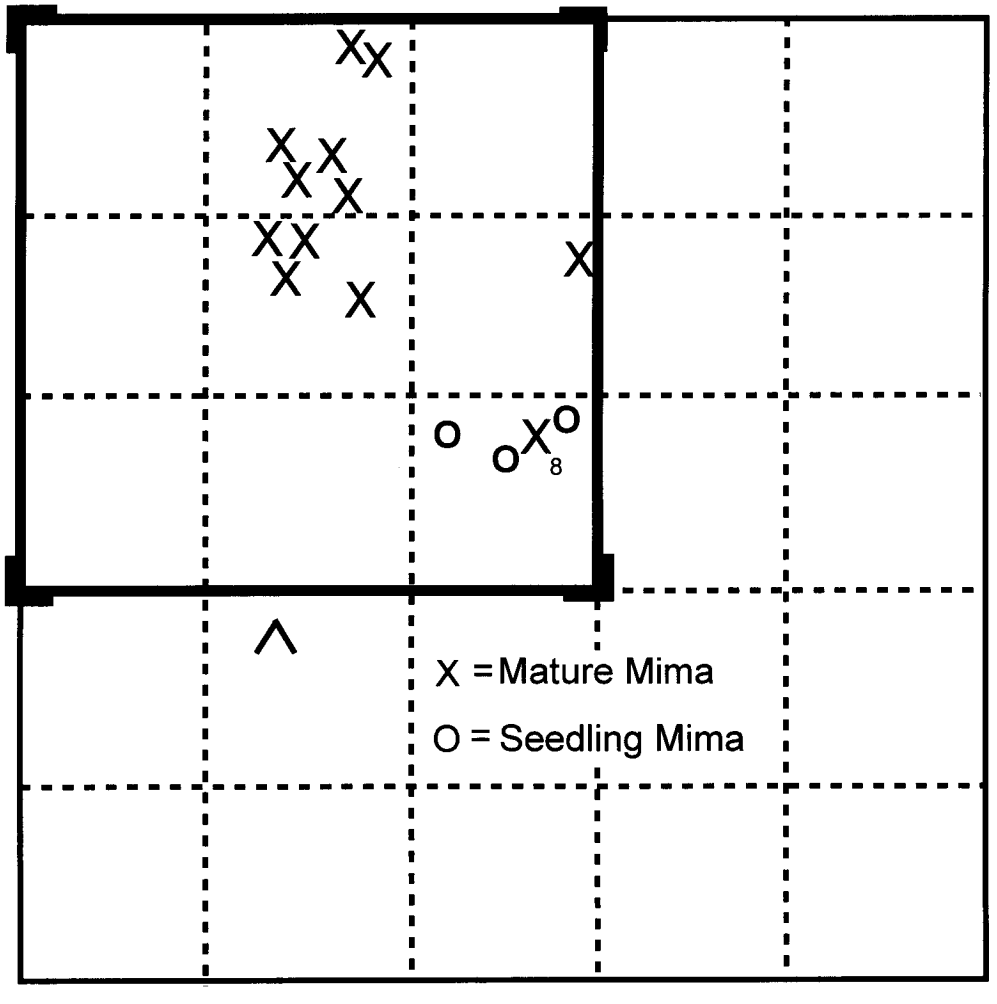


FIG. 11. Clonal map of McKinzie Creek population. Different patterns indicate the 8 unique genotypes given in table 11. Each circle represents asingle ramet, approximating plant size and location in the population. Only those circles with numbers next to them were sampled.

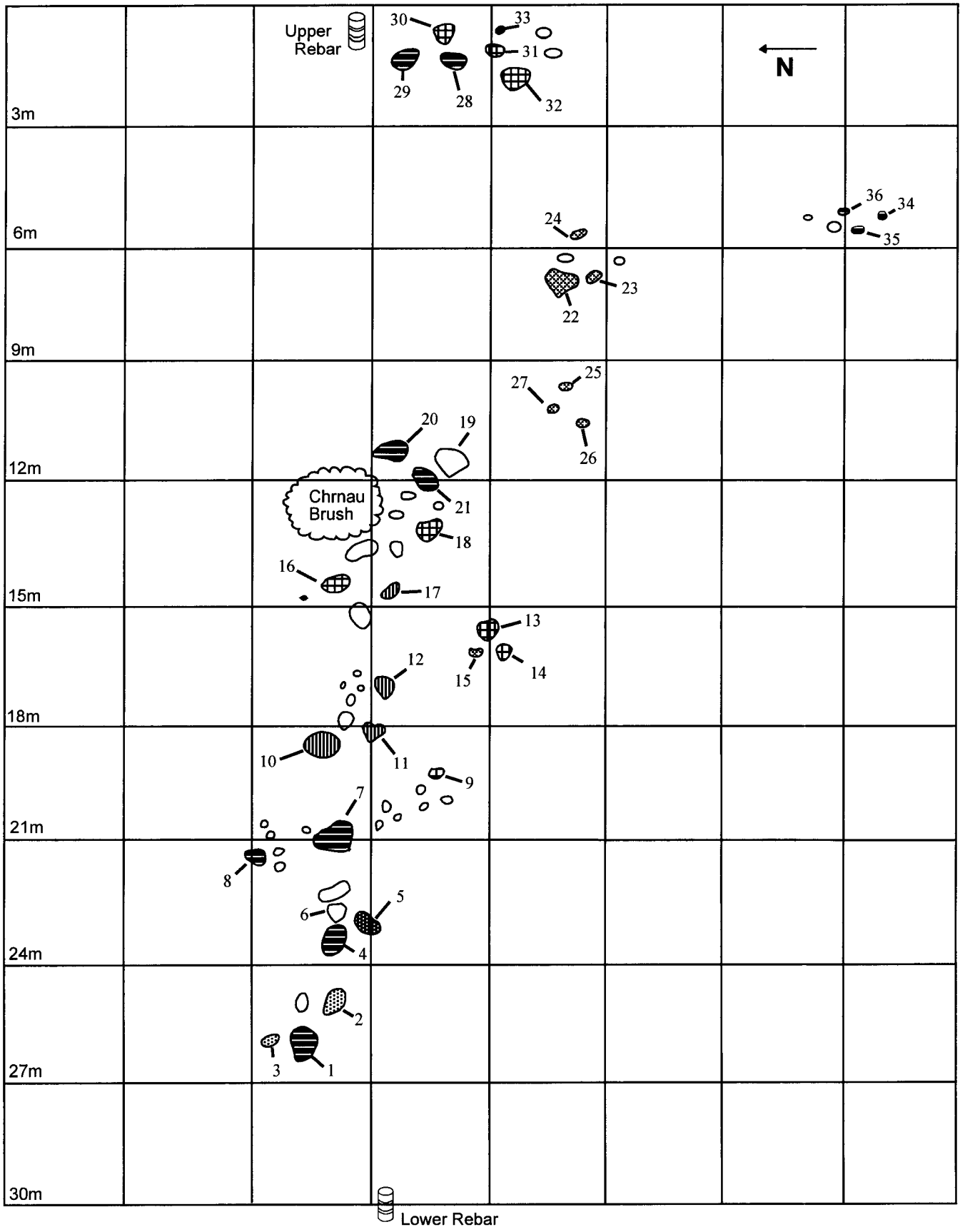


FIG. 12. Long Gulch 8x8 m plot. Different patterns indicate the 9 unique genotypes given in Table 12. Each circle represents a single ramet, approximating plant size and location in the population. Unpatterned ramets are unknown genotypes, due to bad leaf material. Outlined ramets are indicate estimated clonal patches.

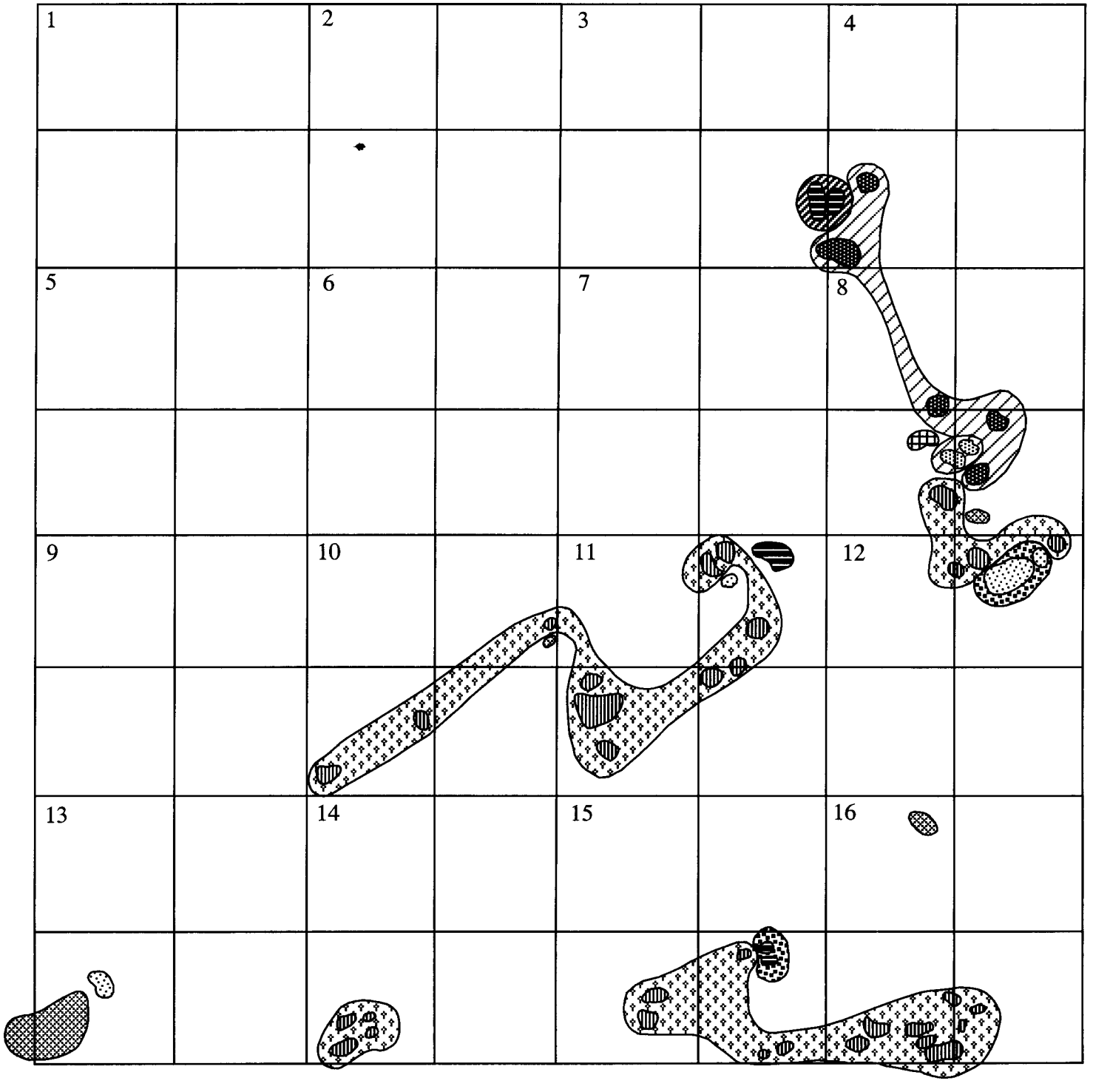


FIG. 13. Lucile Caves rhizome planting site # 1. Leaf samples were collected from all individual stems at each scalp site. Stems are indicated by dots, x's indicate sites were no stems occurred. If multiple stems were present in a site, leaves were collected from 2-4 stems. Numbers indicate different genotypes.

Plot 1

● 109	● 113	×	● ●	×
● 84	×	● 111	×	×
● 32	×	● 86	×	● 111
● 20 ● 113	● 112	● 109	● 109	● 91
● 3	×	● 86	● 29 ● 110	● 69
● 109 ● ● 109 109	92 ● ● ● 92	● 20 ●	● 108 ● 20	● 88 ● 90

FIG. 14. Lucile Caves rhizome planting site # 3. Leaf samples were collected from all individual stems at each scalp site. Stems are indicated by dots, x's indicate sites were no stems occurred. If multiple stems were present in a site, leaves were collected from 2-4 stems. Numbers indicate different genotypes.

Plot 3

● 109	● 86 86 ● ● 86 ● 86	● 85	● 89	×
● 6 28 ● ● 6	● 20 ● 87	×	×	×

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