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# Genetic differentiation of *Microseris pygmaea* (Asteraceae, Lactuceae) studied with DNA amplification from arbitrary primers (RAPDs)

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## SUMMARY

The diploid annual *Microseris pygmaea* is the only Chilean species of the genus and is closely related to *M. bigelovii* and *M. elegans* of California. It must have reached Chile by long-distance dispersal from California and have gone through a single-plant bottleneck before becoming established. Previous data on iso-enzyme alleles and morphology have separated coastal from inland populations and suggested that range expansion took place by stepwise founder effects creating a set of nested monophyletic groups of populations. Here, we test this hypothesis on 10 strains from nine representative populations using nuclear DNA fragments amplified *in vitro* from short arbitrary primers (RAPDs) as characters. We obtained 208 amplification products with 24 primers. Of these, 91 were shared by all strains, 93 were informative. The data confirm that coastal and inland populations form two distinct monophyletic groups, but show relatively weak differentiation within each of these groups indicating some gene flow and recombination among neighbouring populations. Homoplasy in the data due to all possible sources including faults in band interpretation is estimated at about 10%. A cladogram of the two genetically most divergent strains of *M. pygmaea, M. bigelovii* and *M. elegans* shows that each species is monophyletic but does not suggest any closer association between *M. pygmaea* and one of its possible ancestral species.

*Key-words:* Asteraceae, founder event, Lactuceae, long-distance dispersal, *Microseris*, nuclear DNA polymorphisms.

## INTRODUCTION

The small Chilean annual lactucean, *Microseris pygmaea*, is the only representative of its genus in South America (Chambers 1955, 1963). It is very similar to the Californian annuals, especially *M. bigelovii* and *M. elegans*, from which it differs consistently only by its 10-part pappus. The Californian species have a pappus of 5 (or fewer) parts. The

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10-part pappus arises through the addition of a second whorl of five pappus parts in early achene development (Battjes *et al.* 1992). Crosses between *M. pygmaea* and *M. bigelovii* have revealed genetic variation underlying the essentially constant pappus part numbers in both species (Chambers 1963; Bachmann & Chambers 1978). The most likely explanation of the quantitative phenotypes postulates an additively acting system with 1–4 contributing genes in the various genomes (Bachmann *et al.* 1981, 1982, 1984a; Zentgraf *et al.* 1984). In any case, all existing *M. pygmaea* seem to be descended from an individual in which the first allele determining 10 pappus parts became fixed in homozygous condition. It is likely that *M. pygmaea* is the product of long-distance dispersal from California to Chile with subsequent genetic differentiation (Raven 1963; Moore & Lewis 1966; Carlquist 1981,.1983; Bachmann 1983). The fact that coastal populations of *M. pygmaea* share one monomorphic allele for the enzyme glutamate oxalacetate transaminase (Got-2) with all North American species of *Microseris*, while Chilean inland populations are monomorphic for another allele at the locus, supports this interpretation. Presumably, the original colonizer had a five-part pappus and the North American allele of Got-2. The mutation to the first gene determining 10 pappus parts preceded the mutation to the Chilean allele of Got-2. We have tried to fit mutations in other enzyme genes and heritable phenotypic differences among populations into a cladistic scheme of the stepwise evolution of *M. pygmaea* through consecutive founder events during the extension of its range inland and southward (Bachmann *et al.* 1985b; Fig. 3). The genetics of the phenotypic differences are only partially analysed (Bachmann 1991; Bachmann & Chambers 1981; Bachmann *et al.* 1984b, 1985a; Van Heusden 1990; Van Heusden *et al.* 1989), and this scheme is tentative. Certainly, the morphological changes cannot all be accommodated without postulating parallelisms, including independent duplications of the gene that determines 10 pappus parts (Bachmann *et al.* 1985a).

Recently, polymorphisms in the DNA of chloroplasts and nuclei have become available as independent genetic markers for phylogeny and inheritance (Bachmann 1992). There are few polymorphisms among the chloroplast genomes of the annual species of *Microseris* (Wallace & Jansen 1990), and only a few have been found among the populations of *M. pygmaea* (K. Bachmann *et al.* unpublished data). Genetic polymorphisms in the nuclear genome have been looked for using three different methods. Relatively few restriction fragment length polymorphisms (RFLPs) can be detected with cloned homologous genomic DNA probes (W. H. J. Van Houten, unpublished data). Conversely, 'fingerprint loci' detected with simple-sequence oligonucleotides show too much variation for a reconstruction of the genetic differentiation among populations (Van Houten *et al.* 1991). Here we use the polymerase chain reaction (PCR) with arbitrary short primers to amplify stretches of DNA from polymorphic sites (random amplified polymorphic DNAs, RAPDs: Williams *et al.* 1990) and examine the information these polymorphisms provide on the intraspecific differentiation of *M. pygmaea* and its relationship with the two most closely related North American species.

## MATERIALS AND METHODS

The plants used for the assay of intraspecific variation are derived from nine local populations of *Microseris pygmaea* D. Don (Table 1). Some accessions (A92, B95, C30, C37) have been obtained as inbred strains, some as population samples. The heritable variation for phenotypic characters in these populations has been described previously (Bachmann *et al.* 1985a). As in the other autogamous annual species of *Microseris*, most heritable

**Table 1.** Original sources of the inbred strains of *Microseris pygmaea* used in this analysis

| | |
|---|---|
| A92 | Origin unknown (from K. L. Chambers, Oregon State University) |
| B95 | Botanical Garden, Berlin-Dahlem (H. W. Lack accession nr. 275, originally from Botanical Garden Nijmegen) |
| C30 | Santiago Province (K. L. Chambers accession nr. A-221) |
| C37 | O'Higgins Province, Rancagua (from K. L. Chambers) |
| C95 | Prov. de Choapa, Panamericana Norte km 297, north of Huentelauquen (coll. J. Grau, University of Munich) |
| C96 | Prov. de Choapa, Panamericana Norte km 311, near El Teniente (coll. J. Grau) |
| C97 | Prov. de Colchagua, Camino del Cobre (coll. J. Grau) |
| C98 | Prov. de Santiago, Cuesta Barriga (coll. J. Grau) |
| C99 | Region de Coquimbo, Panamericana Norte km 253 (coll. J. Grau) |

variation is among populations. Within populations, there is variation around a recognizable local biotype, which can be propagated in inbred lines. Where there was significant within-population variation, inbred lines have been derived from several field-collected individuals of one population. These lines are designated by small letters (C96b, C98c, C98h). The plants were raised in the greenhouse and strains were maintained by harvesting achenes from spontaneous selfing. Here, we have studied one representative inbred line each from eight populations and two inbred lines from population C98. Some heritable intra-population variability for morphological characters has been demonstrated in this population (Bachmann *et al.* 1985a) as well as hypervariability for DNA fingerprint loci (Van Houten *et al.* 1991). It serves as a control for the assumption that the genetic constitution of a natural population of *M. pygmaea* can realistically be approximated by a randomly chosen inbred strain.

For DNA-isolation, 20 offspring obtained by selfing from a parent plant were raised and leaf material from these plants was pooled. DNA was isolated from freeze-dried leaf material according to the method of Dellaporta *et al.* (1983) with an additional CsCl purification step.

For the comparison of *M. pygmaea* with its two North American relatives, DNA samples from the two most genetically divergent strains of each species were selected on the basis of previous intraspecific comparisons. These are strains D05 and D08 of *M. elegans* Greene ex Gray (Van Heusden & Bachmann 1992a), strains D33b and F11 of *M. bigelovii* (Gray) Sch.–Bip. (Van Heusden & Bachmann 1992b) and A92 and C96b of *M. pygmaea* (this paper).

PCR reactions were performed in 25 µl of the buffer supplied by the Taq polymerase manufacturer (Promega), 0·1 mM dATP, 0·1 mM dGTP, 0·1 mM dCTP, 0·1 mM TTP, 15 ng primer, 50 ng template DNA and 2 units Taq polymerase (Promega). Sequences and sources of primers giving informative amplification products with DNA of *M. pygmaea* are listed in Table 2. Amplification was carried out in a GeneAtaq thermocycler (Pharmacia) programmed for 4 min denaturation at 94°C, followed by 45 cycles of 1 min at 94°C, 1 min annealing at 34°C and 2 min extension at 72°C with a final 5 min extension at 72°C. The entire reaction mixture was taken from under the mineral oil, mixed with the loading dye, Orange G (Maniatis *et al.* 1982), and the amplified products were separated on 1·5% agarose gels by electrophoresis and stained with ethidium bromide (Williams *et al.* 1990; Fig. 3 in Van Heusden & Bachmann 1992a).

Amplification products were listed as discrete character states per strain (present/absent). A branch and bound Wagner parsimony analysis was carried out using PAUP

**Table 2.** Nucleotide sequence (5'–3') of the primers giving informative amplification products with DNA of *Microseris pygmaea*. Primers C1 through C19 were obtained from Isogen Bioscience, Amsterdam, primers with D and E numbers are from the D and E primer kits from Operon Technologies, Almeda, California. Figures in brackets following primer numbers are number of informative amplification products

| | | | | | |
|---|---|---|---|---|---|
| C1 | (5) | 5' CCGGCCGTCA 3' | D1 | (5) | 5' ACCGCGAAGG 3' |
| C2 | (6) | 5' GCGCTCCAAT 3' | D3 | (8) | 5' GTCGCCGTCA 3' |
| C3 | (4) | 5' AACAGCGCCA 3' | D8 | (3) | 5' GTGTGCCCCA 3' |
| C4 | (6) | 5' CACGCGACTA 3' | D10 | (11) | 5' GGTCTACACC 3' |
| C5 | (1) | 5' CGGCTGCTGT 3' | D11 | (1) | 5' AGCGCCATTG 3' |
| C11 | (5) | 5' AGCGAGAAGC 3' | D12 | (5) | 5' CACCGTATCC 3' |
| C15 | (2) | 5' TGCCGGGTGG 3' | D13 | (2) | 5' GGGGTGACGA 3' |
| C16 | (2) | 5' GCGATTGGGG 3' | D15 | (4) | 5' CATCCGTGCT 3' |
| C17 | (3) | 5' CCACAAACGC 3' | D16 | (4) | 5' AGGGCGTAAG 3' |
| C18 | (2) | 5' AGTCTGGCGT 3' | D18 | (4) | 5' GAGAGCCAAC 3' |
| C19 | (5) | 5' AGTCTGGCGT 3' | D20 | (2) | 5' ACCCGGTCAC 3' |
| E4 | (1) | 5' GTGACATGCC 3' | | | |
| E6 | (2) | 5' AAGACCCCTC 3' | | | |

version 3·0 developed by D. L. Swofford (Swofford & Olsen 1990). The bootstrap option was used to run 100 replicates to obtain confidence intervals (Felsenstein 1985). We also used the data to calculate the proportion of shared amplified fragments (F) for each pair of strains. The F values were used to reconstruct a tree using the UPGMA method (Sneath & Sokal 1973; Swofford & Olsen 1990).

## RESULTS

### *Strains of* M. pygmaea

A total of 208 amplification products were obtained with the 24 primers listed in Table 2. Of these, 91 were common to all strains, 12 were absent and 12 present in only one of the strains, the remaining 93 amplification products were informative. The 93 informative characters defined 40 groups of strains (Table 3). Only 11 of these groups are identified by more than one common amplification product. There is a very strong dichotomy between the coastal strains (C95, C96 and C99), a group supported by 17 synapomorphic amplification products, and the remaining, inland strains, supported by 24 shared characters. The distributions of only 19 characters are not compatible with the suggested monophyly of coastal and inland strains. A group of three strains, A92, C30 and C37, is defined by four shared characters and compatible with the distributions of all but 19 of the characters. Four characters (Table 3, group 6) unite the two inbred lines isolated from population C98, one character (Table 3, group 14) unites all inland strains except the two isolates from C98, and only 13 character distributions are not compatible with a monophyletic origin of these two lines. Nineteen and 13 character distributions would correspond to 9·1% and 6·3% homoplasy on the basis of all 208 amplification products, or 10·3% and 7·1%, when autapomorphic markers are omitted.

The groups seen by inspection of the data are reflected in an overall similarity tree based on pairwise comparisons of the percentage of shared amplification products (UPGMA, Table 4, Fig. 1) and in the shortest cladistic tree based on shared amplification products

**Table 3.** Groups of strains of *Microseris pygmaea* defined by common RAPD amplification products. Underlined strain numbers are doubtful assignments ('?' in the data set)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | A92 | C37 | C97 | B95 | C30 | C98c | C98h | C95 | C99 | C96b | 91 |
| 2. | A92 | C37 | C97 | B95 | C30 | C98c | C98h | | | | 24 |
| 3. | | | | | | | | C95 | C99 | C96b | 17 |
| 4. | A92 | C37 | | | C30 | | | | | | 4 |
| 5. | A92 | C37 | C97 | B95 | C30 | C98c | C98h | C95 | | | 3 |
| 6. | | | | | | C98c | C98h | | | | 3 |
| 7. | A92 | C37 | C97 | B95 | C30 | C98c | C98h | | C99 | | 2 |
| 8. | A92 | C37 | C97 | B95 | C30 | C98c | | | | | 2 |
| 9. | A92 | C37 | | B95 | C30 | C98c | C98h | | | | 2 |
| 10. | A92 | | | | | C98c | | | | | 2 |
| 11. | | | C97 | B95 | | | | | | | 2 |
| 12. | | | | | | | | C95 | | C96b | 2 |
| 13. | A92 | C37 | C97 | B95 | C30 | C98c | C98h | | | C96b | 1 |
| 14. | A92 | C37 | C97 | B95 | C30 | | | | | | 1 |
| 15. | A92 | C37 | C97 | | | | | | | | 1 |
| 16. | A92 | C37 | C97 | | | C98c | | | | | 1 |
| 17. | A92 | C37 | C97 | | C30 | | C98h | C95 | C99 | C96b | 1 |
| 18. | A92 | C37 | | B95 | C30 | C98c | | | | | 1 |
| 19. | A92 | C37 | | B95 | C30 | | | | | | 1 |
| 20. | A92 | C37 | | B95 | | | | | | | 1 |
| 21. | A92 | C37 | | | C30 | C98c | C98h | C95 | | C96b | 1 |
| 22. | A92 | C37 | | | C30 | C98c | C98h | | | | 1 |
| 23. | A92 | | C37 | | | | | | | | 1 |
| 24. | A92 | | C97 | | | | | C95 | C99 | C96b | 1 |
| 25. | A92 | | | B95 | C30 | C98c | C98h | | C99 | C96b | 1 |
| 26. | A92 | | | | | | | C95 | C99 | C96b | 1 |
| 27. | | C37 | C97 | B95 | C30 | C98c | C98h | C95 | C99 | | 1 |
| 28. | | C37 | C97 | B95 | C30 | C98c | C98h | | | | 1 |
| 29. | | C37 | C97 | B95 | C30 | | C98h | | | | 1 |
| 30. | | C37 | C97 | | | | | | | | 1 |
| 31. | | C37 | | B95 | C30 | C98c | C98h | | | | 1 |
| 32. | | C37 | | B95 | C30 | C98c | C98h | C95 | C99 | C96b | 1 |
| 33. | | C37 | | B95 | C30 | C98c | | | | | 1 |
| 34. | | C37 | | B95 | | | C98h | | | | 1 |
| 35. | | | C97 | B95 | C30 | | C98h | C95 | C99 | C96b | 1 |
| 36. | | | C97 | B95 | | C98c | | C95 | C99 | C96b | 1 |
| 37. | | | | B95 | C30 | C98c | C98h | C95 | C99 | C96b | 1 |
| 38. | | | | B95 | | C98c | | | | | 1 |
| 39. | | | | B95 | | | | C95 | C99 | C96b | 1 |
| 40. | | | | | | | | C95 | C99 | | 1 |
| 41. | | | | | | | | | C99 | C96b | 1 |

(Fig. 2a), which has a length of 140 steps and a consistency index of 0·686. As seen from the data, the various groups have very different statistical support. The bootstrap consensus tree shown in Fig. 3b retains the few relatively reliable strain associations. It is evident that only the dichotomy between the coastal and inland strains separates two monophyletic subgroups within the species. The two isolates from population C98 form a monophyletic group, but even that group is not very strongly supported. Otherwise none of the groupings is highly significant.

**Table 4.** A matrix of the proportion of amplified DAN fragments (F) shared among 10 different strains of *Microseris pygmaea* used in this study

| | A92 | C37 | C97 | B95 | C30 | C98c | C98h | C95 | C99 | C96b |
|---|---|---|---|---|---|---|---|---|---|---|
| A92 | 1 | | | | | | | | | |
| C37 | 0·91 | 1 | | | | | | | | |
| C97 | 0·81 | 0·82 | 1 | | | | | | | |
| B95 | 0·82 | 0·87 | 0·86 | 1 | | | | | | |
| C30 | 0·89 | 0·94 | 0·80 | 0·88 | 1 | | | | | |
| C98c | 0·86 | 0·88 | 0·78 | 0·87 | 0·88 | 1 | | | | |
| C98h | 0·82 | 0·87 | 0·80 | 0·85 | 0·88 | 0·91 | 1 | | | |
| C95 | 0·61 | 0·60 | 0·58 | 0·61 | 0·61 | 0·61 | 0·63 | 1 | | |
| C99 | 0·62 | 0·60 | 0·58 | 0·61 | 0·63 | 0·61 | 0·65 | 0·89 | 1 | |
| C96b | 0·59 | 0·58 | 0·56 | 0·58 | 0·60 | 0·59 | 0·62 | 0·89 | 0·93 | 1 |

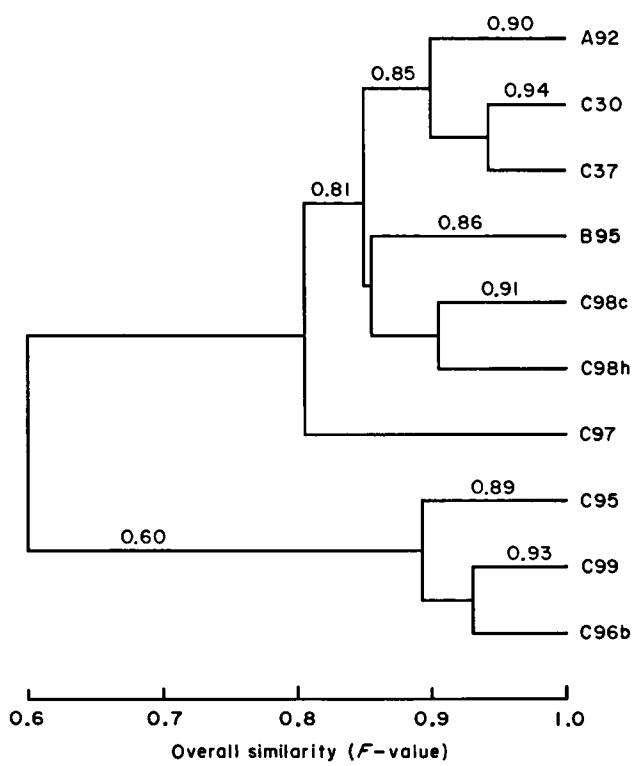


**Fig. 1.** Similarity tree based on the percentage of shared bands (F, numbers above branches) in pairwise comparisons using UPGMA.

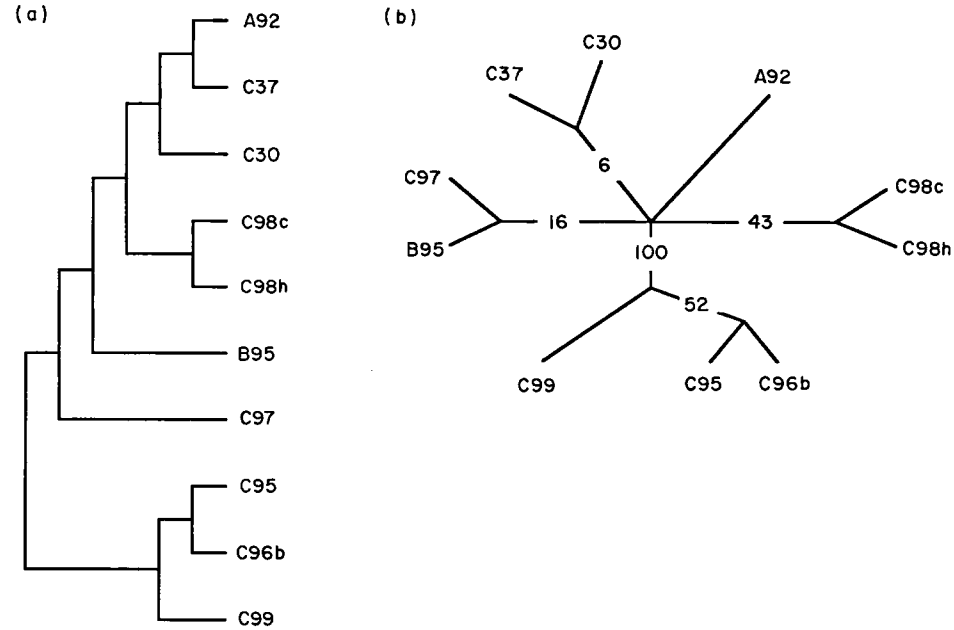


**Fig. 2.** (a) The most parsimonious tree (140 steps) with a consistency index of 0·686 obtained by Wagner parsimony using the branch and bound search option of PAUP; (b) majority rule consensus tree generated by bootstrap analysis using PAUP. Numbers on the branches indicate the percentage of times that the group was found.

*Relationship of* M. pygmaea, M. elegans, *and* M. bigelovii

A comparison of two diverse strains each from *M. pygmaea, M. bigelovii* and *M. elegans* using 22 primers yielded 143 shared amplification products (unique bands were not scored). Of these, 49 were shared by all six strains, i.e. by the three species, 32 were species specific, 12 were present in all but one of the strains, and 50 were informative. Of the 32 species-specific bands, six were markers for *M. elegans*, 12 for *M. bigelovii* and 14 for *M. pygmaea.* Of the two strains per species, the following had autapomorphic absence of amplification products: D05 in *M. elegans* (seven bands), D33b in *M. bigelovii* (one band), and A92 in *M. pygmaea* (four bands). Three pairwise combinations of the three species are possible, and we found five synapomorphic markers for each of these. These data show that the majority of the considerable number of bands shared among all strains of one species is shared by all three species; few are shared among two species only, and there remain an appreciable number of true species-specific bands. While the result is compatible with a monophyletic origin of each of the species, it does not suggest a closer association between *M. pygmaea* and one of the two related species. The result, therefore, contributes little to the search for the nearest relative of the North American ancestor of *M. pygmaea* among the existing populations of *M. bigelovii* and *M. elegans.*

## DISCUSSION

The analysis of intraspecific variation in the three related species, *Microseris pygmaea* (this paper), *M. elegans* (Van Heusden & Bachmann 1992a), and *M. bigelovii* (Van Heusden &

**Table 5.** Comparison of the results of DNA amplification in *M. elegans* (Van Heusden & Bachmann 1992a), *M. bigelovii* (Van Heusden & Bachmann 1992b), and *M. pygmaea* (this paper)

| | *M. elegans* (10 strains) | *M. bigelovii* (13 strains) | *M. pygmaea* (10 strains) |
|---|---|---|---|
| Primers used | 17 | 22 | 24 |
| Products scored | 134 | 194 | 208 |
| Products per primer | 7·9 | 8·8 | 8·7 |
| Common to all strains | 23 (17%) | 44 (23%) | 91 (44%) |
| Informative | 88 (66%) | 118 (61%) | 93 (45%) |

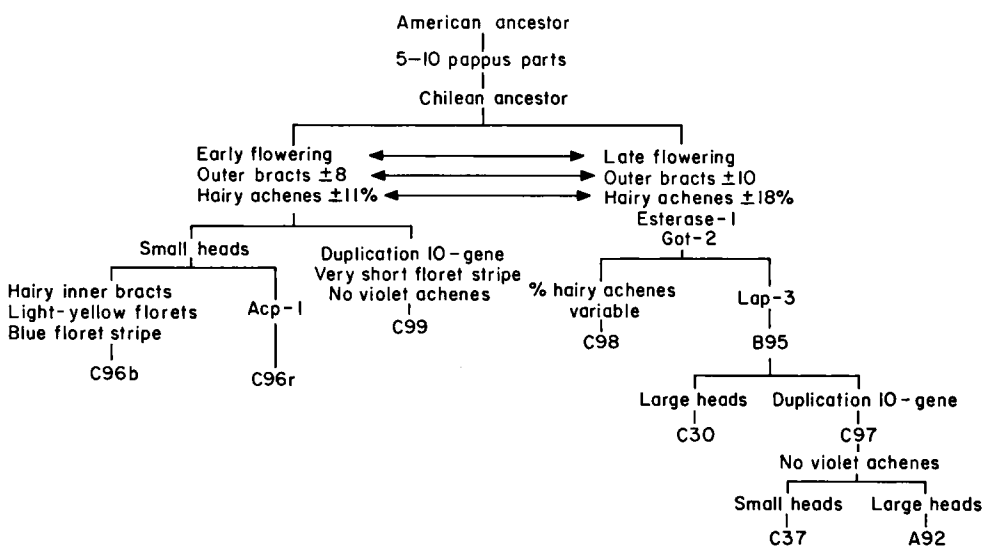


**Fig. 3.** Tentative scheme of the subspecific evolution of *Microseris pygmaea* based on the distribution of fixed enzyme allele differences (acid phosphatase-1, esterase-1, glutamate oxalacetate transaminase-2, leucine aminopeptidase-3) and phenotypic characters with demonstrated heritability. Duplication of a gene determining 10 instead of five pappus parts in *M. pygmaea* is inferred from genetic segregation of quantitative phenotypes. Sources of the data are given in the text.

Bachmann 1992b) using RAPDs has shown some features general to all three species and some subtle differences among their patterns of intraspecific variation. The method is relatively new, and we do not know how safe are the underlying assumptions. The most important of these is the identity of co-migrating amplification products, the random distribution of the many polymorphic sites throughout the genomes, and the quantitative relationship between the number of amplification products and the number of potential sites for amplification in the genome. We have estimated above the proportion of homoplasy due to all reasons in the *M. pygmaea* data to be roughly 10%, which is a tolerable level. As the genomes of the three species differ very little in size (Price & Bachmann 1975; Bennett & Smith 1976), a tentative quantitative comparison may be possible (Table 5). This shows an appreciable increase in the proportion of amplification

products common to all strains of a species from *M. elegans* (17%) and *M. bigelovii* (23%) to *M. pygmaea* (44%) and a correlated decrease in the informative variation among the various strains within a species. Our new data show that most of the bands found throughout one of the species are shared among all three species. In spite of the considerable and readily detected variability of RAPDs, the method obviously also reveals quite a few very conservative markers and may be useful for taxonomy at a level higher than the species level. The remaining, truly species-specific markers show the trend observed for all conservative amplification products: there are few in *M. elegans* and most in *M. pygmaea*. Several factors could explain this trend, among them evolutionary time (*M. pygmaea* may be the youngest species), original population size (only *M. pygmaea* may have originated from a single achene), and ecotypical selection (*M. pygmaea* may have been subject to fewer differential selection pressures than the other species). With the great number of potential markers available through the RAPD method, we can begin to think about tests that may help sort out the influence of these various factors.

We have previously used a cladistic approach to the RAPD data to detect possible subspecific monophyletic groups of populations, which persist due to their genetic isolation from other genotypes. We have rather clear evidence for monophyletic groups of populations within *M. elegans*, while in *M. bigelovii* only local nearest neighbours, such as the disjunct populations on Vancouver Island, might have differentiated from a common origin and in isolation. We orginally derived the idea of an intraspecific evolution by stepwise founder events from the distribution of fixed allelic forms of enzymes and from the morphological variation in *M. pygmaea* (Bachmann *et al.* 1985b, Van Heusden, 1990). One version of the possible population cladogram, based on strain differences under common greenhouse conditions and on the genetics of crosses with a common tester strain of *M. bigelovii*, is shown in Fig. 3. (Note that two different lines from strain C96 were included in the genetic tests, while C95 was not available for the initial crosses). The present data provide an independent test of the postulated relationships.

The most obvious difference between the phenotypic and the DNA data sets is the statistical basis. The few heritable phenotypic differences among the strains can be fitted into a cladistic pattern assuming that the populations are derived from one another by stepwise founder events, but they provide no statistical support for this scenario. The distribution of alternatively fixed enzyme alleles supports such an evolution. The DNA data clearly and strongly support the genetic isolation that exists between the coastal and the inland strains of *M. pygmaea*, but do not support a strict monophyletic (single-achene founder) origin of (and/or absence of gene flow among) populations within either the coastal or the inland subgroups. The evolutionary topology of the shortest tree suggests a linear sequence of origin of the inland populations, but the sequence (C97-B95-C98-C30-C37-A92) does not agree with the most parsimonious sequence for enzyme and phenotype changes, in which population C98 clearly is the least derived genotype (especially on the basis of the genotype for Lap-3; this also agrees with the intermediate geographical location of C98), while population C97 should group with C37 and A92 unless we claim three independent origins of the strong determination (due to gene duplication?) of 10 pappus parts.

The strain distribution of quite a few amplification products is compatible with a single origin of the derived allele of Lap-3, and the apparent disagreement between the enzyme and the RAPD cladograms may yet be resolved. For that, we need more data on alternative fixed alleles. These could be restriction fragment length polymorphisms detected with cloned single copy probes or comparative sequence data.

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