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Genetic analysis of a *Microseris douglasii* (Asteraceae) population polymorphic for an alien chloroplast type*

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Abstract: Recent evidence suggests chloroplast introgression from *Microseris bigelovii* into *M. douglasii*. We have examined 23 plants from a population of *M. douglasii* polymorphic for *M. douglasii* and *M. bigelovii* chloroplast types. All 23 plants were completely homozygous for morphological and RAPD markers, and inbred lines derived by selfing have been used for DNA analysis. Chloroplast RFLP analysis identified 16 plants with *M. bigelovii* chloroplasts and seven with *M. douglasii* chloroplasts. The nuclear genomes of the 16 plants with *M. bigelovii* chloroplasts were examined with 22 primers for RAPD amplification products shared exclusively with *M. bigelovii*. Five of 268 markers appeared to be shared between *M. bigelovii* and one or more of these 16 plants on the basis of their position in gels. Detailed examination of these five amplification products showed that none of them are nuclear DNA from *M. bigelovii*. Very little, if any, nuclear DNA from *M. bigelovii* can be present in *M. douglasii* plants with chloroplasts typical of *M. bigelovii*. The study demonstrates the usefulness of the RAPD technique for screening large numbers of markers to select a few potentially informative ones for rigorous examination.

The annual *Microseris douglasii* (Asteraceae) occurs in western North America and is the most variable among the annual species of the genus (CHAMBERS 1955). Like most of these, it reproduces nearly exclusively by self-fertilization and most plants in nature are highly inbred. In spite of this, there is an apparently random association and distribution of characters throughout the range of the species with virtually no indication of an adaptive component in the distribution of characters or genotypes (BACHMANN & BATTJES 1994, BACHMANN & ROELOFS 1995).

Based on morphology and crossing fertility, CHAMBERS (1955) suggested that *M. douglasii* is a sister species to a group of three other diploid annuals that include *M. bigelovii* (Fig. 1a). Phylogenetic reconstruction with nuclear markers (ROELOFS & BACHMANN 1997) also strongly suggests that *M. douglasii* is a sister group to the

* Dedicated to emer. Univ.-Prof. Dr FRIEDRICH EHRENDORFER on the occasion of his 70th birthday

Fig. 1. Alternative phylogenies for the four diploid annual *Microseris* species. doug *M. douglasii*; doug-1 to doug-4, different chloroplast genotypes found in *M. douglasii*; ele *M. elegans*; big *M. bigelovii*; pyg *M. pygmaea*. A Suggested relationship on the basis of morphology, crossing fertility, microsporangium number and nuclear RAPD markers (CHAMBERS 1955, BATTJES & al. 1994, ROELOFS & BACHMANN 1996). B Phylogeny on the basis of cpRFLP markers (WALLACE & JANSSEN 1990, ROELOFS & BACHMANN 1996). Informative restriction sites are indicated as black bars

three annuals and specifically shows a closer association between *M. bigelovii* and the Chilean *M. pygmaea* within that group. The data of WALLACE & JANSSEN (1990) on restriction site variation in the chloroplast DNA of *Microseris* showed little variation among these annuals. However, if the few polymorphisms are strictly interpreted, they suggest that *M. douglasii* is polyphyletic and that (the chloroplasts of) the three other species have arisen independently within *M. douglasii*. A detailed examination of the chloroplast phylogeny by ROELOFS & BACHMANN (1997) has confirmed this result: four different chloroplast types were found in various populations of *M. douglasii*, sometimes two per population. Two of these chloroplast DNAs are restricted to *M. douglasii*, one is also found in most populations of *M. bigelovii*, and one appears ancestral to the chloroplast types found in *M. bigelovii* and *M. pygmaea* (Fig. 1b). The most likely interpretation of the disagreement between nuclear and chloroplast phylogenies is the assumption that the nuclear phylogeny most closely approximates the organismic evolution of the species, and that chloroplasts of *M. bigelovii* and of a taxon ancestral to it have been transferred in at least two independent events into *M. douglasii*.

The effect ("chloroplast capture" or "chloroplast introgression") has been observed with surprising frequency in diverse groups of angiosperms since chloroplast phylogenies have become available (SOLTIS & KUZOFF 1995, RIESEBERG & al. 1996a). For *M. douglasii*, selective loss of the maternal *M. bigelovii* genome from a diploid hybrid between the two species, possibly during several generations of selfing rather than by backcrossing, seems the most likely origin (ROELOFS & BACHMANN 1997). Artificial hybrids between the two species (CHAMBERS 1955, BACHMANN & HOMBERGEN 1997) do not eliminate one of the contributing genomes in the first generation but produce many lethal and sterile recombinants in later

generations of selfing, which would allow for selection against nuclear DNA markers from one parent.

Under these circumstances we might still expect to detect remnants of an *M. bigelovii* nuclear genome in plants of *M. douglasii* with *M. bigelovii* type chloroplasts. Morphological observations on plants from two of the four populations polymorphic for chloroplast types suggested that (different) nuclear genes for flower color and leaf shape from *M. bigelovii* may be associated with *M. bigelovii* chloroplasts in these populations. ROELOFS & BACHMANN (1995) followed this suggestion and analyzed nuclear and chloroplast markers in a population of *M. douglasii* from Cortina Ridge, California, polymorphic for typical *M. douglasii* and *M. bigelovii* chloroplasts. The analysis revealed only a single plant with *M. bigelovii* chloroplasts in this population so that no statistical comparison could be made. Here, we are repeating this analysis with plants from another population in which more plants with each chloroplast type are available. We shall demonstrate that the examination of many RAPD amplification products eventually did not yield a single one occurring only in *M. bigelovii* and plants of *M. douglasii* with *M. bigelovii* chloroplasts. Whatever the mechanism of chloroplast capture in this case, it must be very efficient in excluding or eliminating the nuclear genome of the chloroplast donor. The study also illustrates the usefulness of RAPDs for quickly and inexpensively screening a large number of genomic sites for markers of a specific type before applying more involved rigorous tests to the few candidate polymorphisms. In this case, an interpretation of the RAPD data alone without added tests would have led to a false conclusion.

Material and methods

Plant material. Mature capitula of two neighbouring *M. douglasii* populations (accession numbers E60 and E63) were collected by JOHANNES BATTJES and KENTON L. CHAMBERS, late spring of 1991 in Solano County, California. These two populations, separated by about 10 km represent one of the extremely rare cases in which substantial gene exchange between populations can be demonstrated (ROELOFS & BACHMANN 1996). Since entire homozygous multi-locus genotypes are shared, this must occur via achene transport between the populations and has the consequence that intra-population variation exceeds that between them. Thus, we treated E60 and E63 as one single population. Single capitula were harvested from 20 plants in Rio Vista (E60.1 to E60.20) and 3 plants in Cook Lane (E63a to E63c). Concerning chloroplast type, E60 was previously identified as a polymorphic population: E60.1 contained typical *M. douglasii* chloroplasts whereas E60.5 and E60.12 contained *M. bigelovii* chloroplasts. All 3 plants from site E63 contained only *M. bigelovii* chloroplasts (ROELOFS & BACHMANN 1997). Offspring of E60.1, .5 and .12 and E63a,b and c are second generation plants raised in 1992/1993, whereas the remaining 17 E60 lines are first generation plants.

Plant germination, planting conditions and biosystematic analysis were performed as previously described by ROELOFS & BACHMANN (1995). Two individually potted plants per plant-derived line were scored for the full set of morphological characters (BACHMANN & BATTJES 1994 Table 2). For RAPD analysis a single leaf of each strain was used to isolate DNA with a minipreparation method described by HOMBERGEN & BACHMANN (1995). Leaves of 12 offspring plants per line were harvested and pooled to isolate enough total DNA for Southern blots.

RAPD analysis. PCR amplifications for RAPD analysis (WILLIAMS & al. 1990, WELCH & McCLELLAND 1990) were performed in a MJ Research (US) thermal cycler as described by ROELOFS & BACHMANN (1995). We used the following random decamer primers (Operon Technologies Inc., US): OPA-5,-7 and -15; OPB-1; OPC-1,-2,-5,-6,-7 and -8; OPD-5; OPF-1 and -10; OPH-3,-4,-12,-13 and -19; OPK-7,-10,-11 and -14. Taq polymerase was provided by HT Biotechnologies (UK). Amplification products were separated on 1.5% agarose gels and visualized by ethidium bromide staining.

Hybridization of RAPD bands. RAPD patterns of interest have been blotted onto "Qiabrane Plus" membrane (Qiagen, Germany) using the vacuum blotting system of Biorad (US). Relevant RAPD bands have been isolated, re-amplified in the presence of ^{32}P and used as probes in hybridization experiments with the blotted original RAPD pattern. Markers that showed promising hybridization patterns have been cloned in the pGEM-T vector (Promega, US) to be further characterized.

Sequencing of RAPD bands. Relevant RAPD clones were further purified with Wizard DNA purification kit (Promega, US) and sequenced on an "Alf Express" automated sequencer (Pharmacia-Biotech, Sweden).

Chloroplast RFLP analysis. DNA isolation from 3 g of fresh leaf material per strain was performed using the maxi preparation method of SAGHAI-MAROOF & al. (1984) modified by ROELOFS & BACHMANN (1995). Approximately 2 μg DNA was digested with each of the restriction enzymes *Dra*I, *Eco*RI, *Eco*RV and *Hinf*I. Southern blotting and hybridization was performed as described by ROELOFS & BACHMANN (1997). Lettuce Sac I cpDNA clones described by JANSEN & PALMER (1987) were used as probes.

Data analysis. RAPD bands were digitally scored as discrete (present/absent) characters using the IS1000 imaging system (Alfa Innotech Corporation, US) in combination with RFLPscan software (Scanalytics, CSPI, US). The data were used as cladistic characters in PAUP 3.1 (SWOFFORD 1993). The sister species *M. bigelovii*, *M. pygmaea* and *M. elegans* were used as outgroup.

Results

Sixteen of 23 plants contain *M. bigelovii* chloroplasts. Based on previous data (ROELOFS & BACHMANN 1997) chloroplast genotype could be readily determined from the hybridization patterns. Accessions E60.1, .2, .3, .4, .6, .7 and .9 contain typical *M. douglasii* chloroplasts. Accessions E60.5, .8, and .10 to .20 as well as E63a,b and c contain chloroplasts typical for *M. bigelovii*.

RAPD analysis reveals inbred lines. Twenty-two primers yielded approximately 268 amplification products, 34 of which were common to all 23 lines. Only 57 polymorphic bands (2–5 per primer) were considered reliably scorable and reproducible. Treating these as cladistic characters at the level of individual plants (lines) within a population should provide information about the genetic relationship among the various homozygous plants that make up the population. Using the heuristic search option of PAUP, 46 shortest trees of 103 steps were obtained (CI=0.485, RI=0.775). Figure 2 shows the 80% majority rule consensus tree of the bootstrap analysis of 200 replicates using the heuristic search option. Much of the cladistic structure in this tree is due to the clear separation of the *M. douglasii* plants from the strains selected to represent the three sister species, *M. elegans*, *M. bigelovii*, and *M. pygmaea*, and the close association between the Chilean *M. pygmaea* with *M. bigelovii* among the Californian relatives. All inbred strains from populations E60 and E63 of *M. douglasii* together form a polytomy.

Fig. 2. 80% majority rule consensus tree (200 bootstrap replicates using PAUP) of 23 plants from population E60/E63 based on 57 polymorphic nuclear DNA markers (RAPDs). Bootstrap value (percentage) for each group is indicated by numbers on the branches. *a* to *c*: polymorphic character states. *a* chloroplast type: B *Microseris bigelovii* type, D *M. douglasii* type, E *M. elegans* type, P. *M. pygmaea* type; *b* percent of peripheral, hairy achenes; *c* average number of pappus parts

Table 1. Genetic identity of the three isolated inbred strains, represented by more than one plant. Dissimilarity: amount of polymorphic bands within each group

Plant no.	Bootstrap value (%)	No. of "synapomorphies"	Dissimilarity
E.3, E.9	98	2	2/57
E.12, E.13, E63a, E63b	91	2	4/57
E.14, E.15, E.19	81	1	1/57

Fig. 3. Original RAPD pattern of primer OPA-15 with the 12 most variable *Microseris douglasii* (doug) lines. B15, B21, E68b, C57a, E63a and E63b are *M. douglasii* plants that contain *M. bigelovii* (big) chloroplasts. The 700 bp band (A15-0.7) has been scored as present in *M. bigelovii* and the other two sister species together with *M. douglasii* plants B15, B21, E68b, E68c, E63a and E63b. *c* control, DNA replaced by H₂O in the PCR reaction; *m* marker, Lambda DNA cut with *Eco*RI and *Hind* III. *ele* *M. elegans*, *pyg* *M. pygmaea*

Fig. 4. Autoradiogram of the hybridization experiment using reamplified A15-0.7 as probe on a Southern blot of the original RAPD pattern (Fig. 2). Only E63a and E63b give a hybridization pattern similar to that of the three sister species. For abbreviations see Fig. 2

This is the result expected for a freely interbreeding population. Since these populations consist of essentially homozygous inbred lines, recent gene flow is excluded, but it is likely that these populations are derived from a single colonization event. Of the 23 lines derived from single plants in the field, groups of two (E60.3 and 9), three (E60.14, 15, and 19), and four (E60.12 and 13 together with E63a and b) are nearly identical in their RAPD patterns (Table 1) and share the same chloroplast types, the same phenotypes for the percentage of peripheral, hairy achenes and the same average numbers of pappus parts. These three groups represent multiple collections of plants belonging to the same inbred line, where the inbred lines are characterized by "synapomorphic" RAPD amplification products. The two morphological characters each show at least three distinct phenotypes (0–0.5%, 8.2–9.6%, 11.7–17.2% peripheral achenes; averages of 3.3–3.5, 3.8–4.1, 4.3–4.8 pappus parts). On the basis of these characters and the chloroplast types, there are at least another five distinct genotypes in the 14 plants in which the RAPD patterns show no further substructure. It is obvious that the three plants from population E63 selected to represent the most distinct morphological types from that population, have genotypes that are also represented in E60. This result confirms our conclusion that the neighbouring populations E60 and E63 are genetically derived from the same base population.

Search for RAPD bands from *M. bigelovii* in *M. douglasii*. In our search for nuclear markers indicative of introgression from *M. bigelovii*, we detected five RAPD bands that were present in *M. bigelovii* and in one or more of the *M. douglasii* plants with *M. bigelovii* chloroplasts on the basis of their identical position after electrophoresis in agarose gels. One of these, RAPD marker F1–1.3, was present in *M. bigelovii* and *M. douglasii* E60.17 and E60.18 (containing *M. bigelovii* cpDNA). This marker was previously used in a mapping study of an artificial hybrid between *M. bigelovii* and *M. douglasii* (BACHMANN & HOMBERGEN 1996) and segregated in a 3:1 ratio. Furthermore, F1–1.3 was associated with a QTL for flower color in linkage group 5 (HOMBERGEN & al., unpubl.). Another RAPD band indicative for introgression from *M. bigelovii* A15–0.7 segregated 3:1 in the mapping study of BACHMANN & HOMBERGEN (1996), but did not show linkage in the map. We checked the homology of these bands by hybridization with the amplification products from *M. bigelovii*. The relevant bands from *M. bigelovii* strain C94 were isolated from the gel, reamplified in the presence of ^{32}P and used as probes on Southern blots of the original RAPD gels. All five markers used as hybridization probes showed patterns that differed from the way in which the corresponding RAPD bands had been scored. Two of the markers, including F1–1.3, hybridized only with *M. bigelovii* amplification products, two hybridized with all accessions without correlation with the chloroplast types, and one amplification product, A15–0.7, which has been scored as present in *M. douglasii* strains B15, B21, E68b, E68c, E63a and E63b (Fig. 3) hybridized with the expected band in E63a and E63b only, but marked the corresponding band in all three sister species (Fig. 4). This amplification product was cloned from C94 and E63a, and 10 clones from each source were sequenced. Eight clones from E63a were identical with nine clones from C94. The remaining three clones showed considerable sequence variation. Fig. 6 shows the hybridization pattern of one of the identical A15–0.7 clones used as a probe on a Southern blot of a gel containing the RAPD pattern of

Fig. 5. Original RAPD pattern of OPA-15 with 18 E60 plants. Accessions E60.5, 0.8, and 0.10 to 0.18 contain *Microseris bigelovii* chloroplasts. A15-0.7 has been scored as present in all plants except for E60.10. *M* marker, Lambda DNA cut with *Eco*RI and *Hind* III

Fig. 6. Autoradiogram of the hybridization experiment using a cloned A15-0.7 marker as probe on a Southern blot of the original RAPD pattern shown in Fig. 5. All 18 E60 plants give a hybridization signal in different intensities

18 plants of population E60 amplified with primer A15 (Fig. 5). As Fig. 5 shows, the clone hybridized with all 18 plants of E60 including those with typical *M. douglasii* chloroplasts (E60.1, 0.2, 0.3, 0.4, 0.6, 0.7, and 0.9; see Fig. 2). Hybridization intensities differ strongly among the plants, but are not correlated with the chloroplast types. None of the five potential RAPD amplification products therefore can be demonstrated to be a remnant of nuclear DNA of *M. bigelovii* introduced together with the *M. bigelovii* chloroplast.

Discussion

The cladogram of some selected strains of *M. bigelovii*, *M. pygmaea*, *M. elegans* and *M. douglasii* shown in Fig. 2 re-confirms the sister group relationship between *M. douglasii* and the three other species that is found whenever nuclear-coded

characters are used. It is implied in the monograph by CHAMBERS (1955: fig. 12) who was the first to disentangle the relationships among the various annual species of *Microseris*, and it has been found again with DNA polymorphisms (VAN HOUTEN & al. 1993, ROELOFS & BACHMANN 1997) and with morphological characters (BACHMANN & BATTJES 1994). BATTJES & al. (1994) have shown that a reduction in the number of microsporangia per anther from 4 to 2 in *M. bigelovii*, *M. pygmaea* and *M. elegans* is a clear morphological synapomorphy for the three species.

The chloroplast genome of Asteraceae species is maternally inherited (CORRIVEAU & COLEMAN 1988, SOLTIS & SOLTIS 1989) in all instances reported to date, except for one case in *Coreopsis* (MASON & al. 1994). The phylogenetic relationship of the chloroplast genomes of the annual diploid *Microseris* species has been fully resolved by ROELOFS & BACHMANN (1997), and can only be superimposed on the nuclear phylogeny, when at least two incidences of chloroplast introgression, one from *M. bigelovii*, one from the ancestor of *M. bigelovii* and *M. pygmaea*, are postulated. Similar inconsistencies between nuclear and chloroplast phylogenies have meanwhile been observed in more than 60 cases throughout the angiosperms (SOLTIS & KUZOFF 1995, RIESEBERG & al. 1996a). They must be the result of ancient hybridizations followed by the elimination of the maternal nuclear genome. Eleven different mechanisms have been proposed for this process (reviewed by RIESEBERG & al. 1996a) but it is difficult to produce convincing proof of the exact mechanism after the fact. The inconsistency between the nuclear and the chloroplast phylogenies implies that at best very small traces of the maternal genome may remain in plants with the foreign chloroplasts. This rigorous elimination suggests a directed process stronger than preferential backcrossing of the original hybrid. Since the annual species of *Microseris* reproduce nearly exclusively by selfing, a process involving repeated backcrossing is particularly unlikely in this case.

RIESEBERG & al. (1996b) suggest that selection rather than chance governs the genomic composition of a hybrid: the genomic composition of the natural hybrid species *Helianthus anomalus* very much resembles that of artificial hybrids between *H. annuus* and *H. petiolaris*. In their genomic analysis 71 to 80% of *H. petiolaris* RAPD markers introgressed at significantly lower than expected frequencies in the artificial hybrids. Epistatic interactions implied by marker associations suggest that only loci from *H. petiolaris* with neutral or favorable interactions with the *H. annuus* background persist.

The selective removal of *M. bigelovii* markers from an original *M. bigelovii* × *M. douglasii* hybrid has also been postulated by us for the chloroplast introgression seen here (ROELOFS & BACHMANN 1997). Artificial hybrids between the two species have been made. Depending on the parental populations involved, the fertility of the F1 hybrids is slightly to severely reduced, and there is consequently a more or less severe selection among the recombinant genotypes (CHAMBERS 1955; BACHMANN & al., unpubl.). We know that there is no immediate elimination of the *M. bigelovii* genome in the F1 hybrid. One such hybrid has been mapped intensively with nuclear DNA markers (BACHMANN & HOMBERGEN 1996) and recombinant inbred strains have been derived from it. The proportion of markers from the two parental species in relation to the fertility of the derived lines is being studied at present in the F5 (HOMBERGEN & al., unpubl.).

Initial suggestions that there might be traces of the nuclear genome of *M. bigelovii* in plants of *M. douglasii* with *M. bigelovii* chloroplasts were based on the leaf shapes and flower colors of such strains of *M. douglasii* (ROELOFS & BACHMANN 1997). In an earlier survey of a population polymorphic for chloroplast types (ROELOFS & BACHMANN 1995), only one in 25 plants had *M. bigelovii* type chloroplasts. Here, we have repeated such an analysis with a population in which seven plants had *M. douglasii* and 16 *M. bigelovii* type chloroplasts. With this better comparative basis, we have found five RAPD amplification products in 268 obtained with 22 primers that had distribution patterns suggestive of nuclear introgression. As it turned out, a detailed analysis of the five amplification products did not support this suggestion for any of them.

The problems involved in using RAPDs as nuclear DNA markers and the safeguards necessary for their application have been discussed repeatedly (HADRY & al. 1992; BACHMANN 1992, 1994; CLARK & LANIGAN 1993; WHITKUS & al. 1994; RIESEBERG 1996b; ROELOFS & BACHMANN 1997). The great advantage of RAPDs is the possibility to survey rather quickly and inexpensively large numbers of markers more or less randomly distributed all over the genome and therefore potentially linked with any region or locus of interest. The fact that bands at the same position in a gel are assumed to be identical amplification products always introduces false interpretations. These may introduce acceptable noise in the data, as in the phylogenetic applications at low taxonomic levels, especially at the level of clones or inbred lines (discussed by BACHMANN 1994), or they may not permit conclusions without further investigation. The search for markers for nuclear introgression here is an example for the latter. When the argument rests on a very small number of pre-selected RAPD amplification products, the homology and distribution of these must be independently verified. The advantage of RAPDs lies in the fact, that the preselection does not require such elaborate methods, their disadvantage in the possibility that the few remaining markers turn out to be false positives, as here.

The present study rather than elucidating the mechanism of chloroplast introgression, in fact has added a complicating aspect. Population E60/E63 is polymorphic for plants with two types of chloroplasts. The sequence differences between these are large enough so that the parallel origin of the *M. bigelovii* type chloroplast in *M. douglasii* from an *M. douglasii* chloroplast can be discounted as well as an ancient inherited chloroplast polymorphism that has been sorted out only in the ancestors of the present population. Since on the basis of the nuclear genomes the plants in population E60/E63 look like surviving recombinant inbred lines of a past hybridization event, it is very puzzling that different lines should contain one or the other type of chloroplast. While the results of "chloroplast introgression" turn up ever more often, the precise mechanism in an individual case still needs to be elucidated. We expect that the detailed analysis of artificial hybrids will be the most promising approach.

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References

- BACHMANN, K., 1992: Nuclear DNA markers in angiosperm taxonomy. – *Acta Bot. Neerl.* **41**: 369–384.
- 1994: Molecular markers in plant ecology. – *New Phytologist* **126**: 403–418.
- BATTJES, J., 1994: Variability in a predominantly self-fertilizing annual with fragmented distribution, *Microseris douglasii* (Asteraceae, Lactuceae). – *Biol. Zentralblatt* **113**: 69–95.
- HOMBERGEN, E. J., 1996: Mapping genes for phenotypic variation in *Microseris* (Lactuceae) with molecular markers. – In CALIGARI, P. D. S., HIND, D. J. N., (Eds): *Compositae: biology & utilization*, **2**, pp. 23–43. – Richmond: Royal Botanical Gardens, Kew.
- ROELOFS, T. F. M., 1995: Phenotypic plasticity and parent/offspring regression of characters in inbred strains of *Microseris douglasii* (Asteraceae, Lactuceae). – *Biol. Zentralblatt* **114**: 67–82.
- BATTJES, J., CHAMBERS, K. L., BACHMANN, K., 1994: Evolution of microsporangium numbers in *Microseris* (Asteraceae, Lactuceae). – *Amer. J. Bot.* **81**: 641–647.
- CHAMBERS, K. L., 1955: A biosystematic study of the annual species of *Microseris*. – *Contrib. Dudley Herbarium* **4**: 207–312.
- CLARK, A. G., LANIGAN, C. M. S., 1993: Prospects for estimating nucleotide divergence with RAPDs. – *Molec. Biol. Evol.* **10**: 1096–1111.
- CORRIVEAU, J. L., COLEMAN, A. W., 1988: Rapid screening method to detect potential biparental inheritance of plastid DNA and results over 200 angiosperm families. – *Amer. J. Bot.* **75**: 1443–1458.
- HADRY, H., BALICK, M., SCHIERWATER, B., 1992: Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. – *Molec. Ecol.* **1**: 55–63.
- HOMBERGEN, E. J., BACHMANN, K., 1995: RAPD mapping of 3 QTLs determining trichome formation in *Microseris* Hybrid H27 (Asteraceae, Lactuceae). – *Theor. Appl. Genet.* **90**: 853–858.
- JANSEN, R. K., PALMER, J. D., 1987: Chloroplast DNA from Lettuce and *Barnadesia* (Asteraceae): structure, gene localization, and characterization of a large inversion. – *Curr. Genet.* **11**: 553–564.
- MASON, R. J., HOLSINGER, K. E., JANSEN, R. K., 1994: Biparental inheritance of the chloroplast genome in *Coreopsis* (Asteraceae). – *J. Heredity* **85**: 171–173.
- RIESEBERG, L. H., 1996: Homology among RAPD fragments in interspecific comparisons. – *Molec. Ecol.* **5**: 99–105.
- WHITTON, J., LINDER, C. R., 1996a: Molecular marker incongruence in plant hybrid zones and phylogenetic trees. – *Acta Bot. Neerl.* (in press).
- SINERVO, B. C., LINDER, C. R., UNGERER, M. C., ARIAS, D. M., 1996b: Role of gene interactions in hybrid speciation: evidence from ancient and experimental hybrids. – *Science* **277**: 741–745.
- ROELOFS, D., BACHMANN, K., 1995: Chloroplast and nuclear DNA variation among homozygous plants in a population of the autogamous annual *Microseris douglasii* (Asteraceae, Lactuceae). – *Pl. Syst. Evol.* **196**: 185–194.
- 1997: Comparison of chloroplast and nuclear phylogeny in the autogamous annual *Microseris douglasii* (Asteraceae, Lactuceae). – *Pl. Syst. Evol.* **204**: 49–63.
- SAGHAI-MAROOF, M. A., SOLIMAN, K. M., JORGENSEN, R. A., ALLARD, R. W., 1984: Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. – *Proc. Natl. Acad. Sci. USA* **81**: 8014–8018.
- SOLTIS, D. E., KUZOFF, R. K., 1995: Discordance between nuclear and chloroplast phylogenies in the *Heuchera* group (Saxifragaceae). – *Evolution* **49**: 727–742.

- SOLTIS, P. S., 1989: Allopolyploid speciation in *Tragopogon*: insights from chloroplast DNA. – Amer. J. Bot. **76**: 1119–1124.
- SWOFFORD, D. L., 1993: PAUP: phylogenetic analysis using parsimony, version 3.1. Formerly distributed by Illinois Natural History Survey, Champaign, Illinois.
- VAN HOUTEN, W. J. H., SCARLETT, N., BACHMANN, K., 1993: Nuclear DNA markers of the Australian tetraploid *Microseris scapigera* and its North American diploid relatives. – Theor. Appl. Genet. **87**: 498–505.
- WALLACE, R. S., JANSEN, R. K., 1990: Systematic implications of chloroplast DNA variation in the genus *Microseris* (Asteraceae: Lactuceae). – Syst. Bot. **15**: 606–616.
- WELSH, J., MCCLELLAND, M., 1990: Fingerprinting genomes using PCR with arbitrary primers. – Nucleic Acids Res. **18**: 7213–7218.
- WHITKUS, R., DOEBLEY, J., WENDEL, J. F., 1994: Nuclear DNA markers in systematics and evolution. – PHILLIPS, R. L., VASIL, I. K., (Eds): DNA-based markers in plants, pp. 116–141. – Dordrecht: Kluwer.
- WILLIAMS, J. G., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A., TINGEY, S. V., 1990: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. – Nucleic Acids Res. **18**: 6531–6535.

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