UvA-DARE (Digital Academic Repository)

Intraspecific evolution of *Microseris pygmaea* (Asteraceae, Lactuceae) analyzed by cosegregation of phenotypic characters (QTLs) and molecular markers (RAPDs)

van Houten, W.; van Raamsdonk, L.; Bachmann, K.

DOI

[10.1007/BF00937858](https://doi.org/10.1007/BF00937858)

Publication date

1994

Published in

Plant Systematics and Evolution

[Link to publication](#)

Citation for published version (APA):

van Houten, W., van Raamsdonk, L., & Bachmann, K. (1994). Intraspecific evolution of *Microseris pygmaea* (Asteraceae, Lactuceae) analyzed by cosegregation of phenotypic characters (QTLs) and molecular markers (RAPDs). *Plant Systematics and Evolution*, 190, 49-67. <https://doi.org/10.1007/BF00937858>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)

Intraspecific evolution of *Microseris pygmaea* (*Asteraceae*, *Lactuceae*) analyzed by cosegregation of phenotypic characters (QTLs) and molecular markers (RAPDs)

WIM VAN HOUTEN, LEO VAN RAAMSDONK, and KONRAD BACHMANN

Received May 24, 1993

Key words: *Asteraceae*, *Microseris pygmaea*. — RAPDs, QTLs, populations, hybrid, species formation.

Abstract: The Chilean annual, *Microseris pygmaea*, has differentiated in distinct coastal and inland series of populations after long-distance dispersal from western North America. Two plants from the most diverse biotypes were crossed, a large F_2 was raised and analysed for segregation of 30 phenotypic characters. Segregation of molecular markers (47 RAPDs, 1 RFLP, 2 isozymes) was determined in a subpopulation of 45 plants which include all extremes for the phenotypic characters. 32 marker/character cosegregations were significant at the 1% level in t-tests between dominant and homozygous recessive marker genotypes. Considering linkage among markers and pleiotropy of certain marker loci, the number of independent quantitative trait loci (QTLs) is reduced to about 18. Interactions among 2 or 3 QTLs affecting one character have been characterized. The phenotypic differentiation of *M. pygmaea* during its evolution from a single founder individual begins to be understood at the level of single-gene mutants.

The small diploid annual lactucean *Microseris pygmaea* D. DON ($2n = 18$) of central Chile is the only member of its genus in South America. Its nearest relatives occur in western North America (CHAMBERS 1955) and it is very likely that *M. pygmaea* has evolved in Chile after long distance dispersal from North America. The possession of a 10-part pappus distinguishes *M. pygmaea* consistently from its nearest North American relatives which have five (or fewer) pappus parts (CHAMBERS 1955, 1963). Genetic analysis of this character suggests that all *M. pygmaea* have arisen from a single plant which was homozygous for an allele that adds a second whorl of pappus parts (BATTJES & al. 1992). The comparison of geographically distinct populations of *M. pygmaea*, differing in isozyme and morphological characters has led to the tentative reconstruction of the stepwise differentiation of the species during the expansion of its range from its point of origin on the Chilean coast (BACHMANN & al. 1985 a, VAN HEUSDEN 1990). This intraspecific phylogeny has recently been tested using polymorphic DNA fragments amplified from arbitrary primers (RAPDs, WILLIAMS & al. 1990, VAN HEUSDEN & BACHMANN 1992). The

molecular data have confirmed the existence of two, genetically independent evolutionary lines within the species, one consisting of coastal, the other one of inland populations.

Biotypes from the populations at the endpoints of these two evolutionary branches, represented by inbred lines C 96b (coast) and A 92 (inland), differ in most of the characters that have become genetically fixed in *M. pygmaea* during its differentiation in Chile. This provides us with the unique opportunity to cross the two and obtain an intraspecific hybrid that is heterozygous for the ancestral and derivative alleles of many genes involved in the differentiation of the species. The complex segregation of this hybrid (strain E 33) has been studied. The heritability and segregation of some meristic characters up to the F₃ generation has been described by BACHMANN (1991). Most of the characters show quantitative segregation. In order to find individual genes and alleles, we have tried to make use of molecular markers. We have found few allozyme polymorphisms (BACHMANN & al. 1985 b) and few restriction fragment length polymorphisms (RFLPs) using genomic low-copy-number probes cloned from one of the strains of *M. pygmaea* (VAN HOUTEN, unpubl.). Conversely, DNA fingerprint patterns detected with simple synthetic oligonucleotides proved to be hypervariable in *M. pygmaea* so that their use as segregating markers is severely limited (VAN HOUTEN & al. 1991). In the present paper we report on the segregation of RAPDs in hybrid E 33 and their use as molecular markers for genes determining the phenotypic differences between the parental strains.

Material and methods

Plant material. Strains A 92 and C 96b are maintained as inbred lines from individual field collected plants. Strain A 92 was collected near Santiago di Chile (exact locality unknown) and obtained from K. L. CHAMBERS. Strain C 96b is one of two biotypes derived from a population sample collected in 1980 by J. GRAU at km 311, Panamericana Norte between Huentelauquen and El Teniente (Chile: IV. Región, de Coquimbo, Prov. de Choapa). Strains A 92 and C 96b have been characterized previously (BACHMANN & al. 1985 b). Plants from these two strains were crossed in 1983 by K. L. CHAMBERS to obtain an F₁ hybrid (hybrid strain E 33). The hybrid was raised in 1986/87. This hybrid was selfed, and an F₂ of 300 plants was raised and scored for a variety of phenotypic characters in 1987/88. In 1988/89, selected F₃ offspring families were raised in order to determine the heritability of the character differences found in the F₂. Inbred lines derived from F₂ plants with extreme values for the various quantitative characters were selected and a subsample of 45 lines representing the full phenotypic variation found in the F₂ was raised for DNA isolation and linkage analysis.

DNA isolation. For each line, 20 offspring by selfing from a parental F₂ plant were raised and leaf material from these F₃ plants was pooled for DNA isolation to represent the genotype of the F₂ parent. DNA was isolated as previously described (VLOT & al. 1992) following the method of DELLAPORTA & al. (1983). The CTAB precipitation step was omitted, and instead of a phenol purification DNA was purified in a CsCl gradient.

PCR amplification. DNA was amplified according to the method of WILLIAMS & al. (1990). Table 2 lists the primers used for amplification. Reactions were performed in a 25 µl volume in buffer supplied by the manufacturer (Sphaero Q), 0.2 units Supertaq (Sphaero Q) were used for each reaction. A Gene Ataq thermocycler (Pharmacia) was programmed to execute 45 cycles of 1 min at 94 °C (strand separation), 1 min at 36 °C (primer annealing) and 2 min at 72 °C (chain elongation). Reaction products were resolved on 1.5% agarose

(BRL) gels. In a few cases, absence or presence of a band could not be determined with certainty after repeated independent amplification reactions. Results from these samples were excluded from data analysis. 240 arbitrary 10-basepair primers (Operon kits A, B, D, E, F, U, V, W, X, Y, Z, and Isogen Bioscience kit C) were screened for the generation of polymorphic amplification products from the DNA of strains A92 and C96b. Of these 240 primers, 34 primers consistently revealed polymorphisms between the two strains and were used to determine the segregation in the 45 selected F₂ offspring lines.

Quantitative traits. Descriptions of the quantitative traits that show significant associations with single-gene markers are summarized in Table 1. Most of the traits have been scored on arbitrary scales by inspection. In each case, the same trait has been scored independently by several persons to check on the reliability of the estimates. For the characters in question this method of converting visible differences into quantitative scores is preferable to quantitative measurements. Most characters are subject to various sources of non-genetic variation, especially age-related variation within a plant. Repeated scoring is more important than the precision of the individual determination. Heritability determinations by parent/offspring regression (BACHMANN 1991, and unpubl. data) have shown that scores are reliable quantitative characters.

Data analysis. A total of 47 RAPD bands was scored (Table 2). The data were combined with previously determined segregation data for 2 isozyme loci (Esterase-1, Glutamate-oxalacetate transaminase-2, BACHMANN & al. 1985 b), one RFLP marker (an EcoRV fragment polymorphism detected with probe p12A, an anonymous genomic PstI clone from *M. pygmaea* C96b), 4 DNA fingerprint loci detected with the oligonucleotide probe (GATA)₄ (VAN HOUTEN & al. 1991) and 2 single-gene morphological characters, hairy inner phyllaries (*hip*: VAN HEUSDEN & al. 1989) and violet fruit wall (*via*). Linkage among single-gene markers was calculated using the computer package JoinMap version 1.1 (STAM 1993). Linkage groups were constructed and maps were created according to Haldane's function using lod scores and formulas indicated in Fig. 1. Deviations from expected segregation ratios were tested with a chi-square test.

Linkage between single-gene markers and loci determining phenotypic traits (quantitative trait loci: QTLs) was determined by sorting the F₂ test population into two groups corresponding to the 3 : 1 (RAPDs) or 1 : 2 : 1 (RFLP 12 A) segregation of the markers and testing for significant differences of the average quantitative trait values associated with the marker genotypes.

Results

RAPD variation. The segregation patterns of all markers are summarized in Table 2. Most of these confirm closely to the expected 3 : 1 segregations. Two of the markers with distorted segregation ratios are linked (D 5.3 and U 10.2, see below).

Linkage among single-gene markers. Linkage groups for the markers are shown in Fig. 1, which is based on linkages with lod scores of 2.4 or higher. A few linkages with lod scores of 2.0 have been added when they were supported by common linkage with phenotypic characters (thinner lines in Fig. 1). The nine linkage groups, a through i, shown in Fig. 1 are based on 33 of the 47 RAPDs, one isozyme, 1 fingerprint locus and the two one-locus morphological markers, and comprise 366 centiMorgan. This corresponds probably to about one third of the map length for *M. pygmaea*. Linkage groups a through i therefore are not likely to correspond to the nine chromosomes in the haploid *Microseris* genome.

Quantitative traits. The parental values and the F₂ average and range for the 16 quantitative traits described in Table 1 are shown in Table 3. Some of the F₂ distributions deviate markedly from the expected distribution of a quantitative trait

Table 1. Description of phenotypic characters cosegregating with marker loci

---

Determined by daily inspection:	
HAIRDATE	time in days after planting when glandular trichomes were first seen on the lamina of a rosette leaf. Related to switch from vegetative to flowering rosette. The same trichomes may appear independently on the leaf tip or the leaf teeth much earlier in the vegetative rosette.
BUDDATE	time in days after planting when first (central) capitulum bud is visible.
BUD-HAIR	time in days between appearance of hairs on leaf blade and appearance of first capitulum bud.
Determined by repeated scoring of leaves and buds:	
VIOLPET	anthocyanin colouration in the petioles, scored 183 days after germination, on a scale from 0 (petioles green) to 3 (strong red colour). Average of 3 independent scores.
HIPINT	density of trichomes on the inner involucre bracts (phyllaries), on a scale from 0 (no trichomes) to 25 (high density of trichomes).
REDPHYL	background anthocyanin colouration on the inner phyllaries (not anthocyanin stripe on midrib); on a scale from 0 (green) to 7 (strong violet); if present, it is accentuated by sunlight.
Determined on flowering head at anthesis:	
FLVSPHY	relative length of florets and phyllaries scored on the first day of anthesis on a scale from 2 (showy heads: phyllaries clearly shorter than florets) to 8 (phyllaries clearly longer than florets).
FLOWCOL	basic colour of the floret ligules on a scale from 0 (nearly white) to 9 (dark yellow).
FLSTCOL	coloured stripe on ligules of outer florets: colour, scored on several heads on a scale from 0 (blue) to 10 (red), 5 = brown.
FLSTLGT	coloured stripe on ligules of outer florets: length, on a scale from 0 (absent) to 10 (stripe extends the full length of the ligule).
FLSTINT	coloured stripe on ligules of outer florets: colour intensity, on a scale from 0 (absent) to 10 (very strong).
Determined on ripe fruiting heads:	
SCAPLGT	length of mature scape in cm, average from the first 10 flower heads
INNPHYL	average number of inner phyllaries on first 10 mature heads. Partially dependent on flower head size (achenes/head). Canalized to 13 (minimum in A 92, maximum in C 96b).
ACHENES	average number of achenes per head in first 10 ripe flower heads (34 to 94).
PCTHAIR	percent peripheral, hairy achenes per flower head, the most peripheral achene morph on each capitulum (5% to 25%).
VIOLINT	intensity of the violet spotting on peripheral, hairy and non-hairy light-coloured achenes. Scored by inspection (0, absent; 1, single spot on at least one achene; 6, many small spots; 9, strong large blotches of violet colour; 10, uniform violet colour). Correlated with the percent of peripheral light achenes expressing the character.

Table 2. List of single-gene markers segregating in *Microseris pygmaea* interpopulation hybrid E 33. RAPD amplification products are named for the primer kit (see material and methods); the length of the amplification product and the primer sequence are shown. GATA markers are DNA fingerprint loci labelled with the oligonucleotide (GATA)₄. An RFLP has been detected with genomic probe p 12A. Est-1 and Got-2 are isoenzyme loci, *hip* and *via* are morphological markers. Parents contributing dominant markers are indicated as A or C (strains A 92 or C 96b). Asterisks mark significant deviations from 3 : 1 segregation (critical χ^2 value df = 1: 3.84; P = 0.05)

Marker	Band length (bp)	Sequence	Segregation	χ^2
A 1.1	600	CAGGCCCTTC	A 33:12	0.066
A 1.2	550		C 31:14	0.90
A 17	600	GACCGCTTGT	A 27:18	5.4*
A 19	550	CAAACGTCGG	C 34:11	0.0074
B 1	1300	GTTTCGCTCC	C 27:17	4.36*
B 10.1	600	CTGCTGGGAC	A 36:9	0.60
B 10.2	450		A 33:10	0.070
D 3	1000	GTCGCCGTCA	C 30:15	1.66
pD 5.1	550	TGAGCGGACA	C 43:2	10.14*
D 5.2	400		C 17:8	1.25
D 5.3	200		C 28:17	3.92
D 12	600	CACCGTATCC	A 36:9	0.60
D 18.1	1600	GAGAGCCAAC	A 32:13	0.36
D 18.2	1000		C 35:10	0.81
E 17.1	400	CTACTGCCGT	A 32:13	0.36
E 17.2	550		C 31:14	0.90
E 18.1	500	GGACTGCAGA	A 32:13	0.36
E 18.2	250		A 34:11	0.0074
F 4	1200	GGTGATCAGG	C 32:11	0.0078
F 9	1000	CCAAGCTTCC	A 32:12	0.12
U 8.1	1100	GGCGAAGGTT	A 30:13	0.63
U 8.2	1000		C 28:14	1.55
U 10.1	1450	ACCTCGGCAC	A 21:21	14*
U 10.2	1300		C 24:18	7.14*
U 14.1	800	TGGGTCCCTC	A 35:10	0.19
U 14.2	450		A 31:14	0.90
U 17	400	ACCTGGGGAG	C 36:7	1.74
V 1	1000	TGACGCATGG	C 34:11	0.0074
W 7	1300	CTGGACGTCA	C 33:11	0
W 9	900	GTGACCGAGT	A 32:11	0.0078
W 20	1000	TGTGGCAGCA	A 20:23	18.61*
X 1	300	CTGGGCACGA	C 35:9	0.48
X 4	900	CCGCTACCGA	A 34:11	0.0074
X 7	800	GAGCGAGGCT	A 38:7	2.14
Y 2.1	350	CATCCCCGCA	C 31:14	0.90
Y 2.2	700		A 34:11	0.0074
Y 4	1100	GGCTGCAATG	A 33:10	0.070
Y 6	600	AAGGCTCACC	C 34:11	0.0074
Y 17	1200	GACGTGGTGA	A 28:13	0.98
Z 16	1400	TCCCCATCAC	C 36:8	1.08

Table 2 (continued)

Marker	Band length (bp)	Sequence	Segregation	chi ²
C2	800	GCGCTCCAAT	A 34:11	0.0074
C3.1	500	AACAGCGCCA	A 35:10	0.19
C3.2	400		A 30:15	1.66
C4.1	600	CACGCGACTA	A 30:13	0.63
C4.2	500		A 33:10	0.070
C11	1000	AGCGAGAAGC	C 33:12	0.067
C17	500	CCACAAACGC	A 35:10	0.19
GATA-1	5000		A 37:6	2.80
GATA-2	15000		A 30:14	1.09
GATA-3	3000		C 21:22	15.70*
GATA-4	6000		C 33:10	0.070
p12A	4000/5000		12:24:8	1.09
EST-1			13:19:11	0.77
GOT-2			12:25:7	1.95
<i>hip</i>			C 36:8	1.09
<i>via</i>			C 36:8	1.09

with additive multifactorial inheritance, i.e., a normal distribution between the parental means and centred on the average between the parental values. The most common deviation is a significant and heritable transgressive segregation, i.e., the occurrence of F_2 individuals with character values outside the parental range. In addition, the F_2 means in some cases exceed the parental mean due to heterosis (FLSVSPHY; FLSTINT). Deviations from the expected mean may also be due to a non-normal, strongly skewed distribution of the F_2 values (BUD-HAIR) or to distributions with two peaks near the upper and lower limit (REDPHYL, FLSTLGTH). These latter suggest underlying 3:1 segregations of main genes, but the details in both cases are more complex (see below).

Linkage of phenotypic traits to molecular markers. Since we analysed some 30 quantitative traits that could be determined by at least 50 to 100 loci with appreciable effects, cosegregation between markers and traits is regularly found. Associations between markers and traits cover the entire range from close linkage between a marker and a main gene affecting a quantitative trait to associations at the limit of statistical significance. The number of significant associations (not corrected for linkage or pleiotropy) depended strongly on the statistical criterion. At a level 5% probability, we found 116 associations involving 30 characters, at 1% probability there were 32 associations with 16 characters, at 0.1% 10 associations with 8 characters, at 0.01% 5 associations with 4 characters. Table 4 lists the cosegregation at a significance level of 1% or less. While most of the associations at the 5% level undoubtedly also are true biological effects, there will also be associations due to chance sampling. The essential results of this study can be illustrated with the data of Table 4.

Main effect genes. Two of the characters (HIPINT, VIAINT) could be scored either as qualitative single-gene effects by presence or absence (genes *hip* and *via*)

Fig. 1. Partial linkage map of the single-gene markers in *Microseris pygmaea* interpopulation hybrid E33. Most markers are RAPDs, *hip* and *via* are single gene morphological characters, *Est-1* is an isozyme locus, and *GATA-2* is a DNA fingerprint locus. Details on markers and mapping are given in the text. The nine linkage groups (a) through (i) do not necessarily correspond with the 9 chromosomes in the haploid set

or as quantitative effects by the degree of expression when present. A similar effect can be seen for flower colour (*FLOWCOL*), if the plants are separated into a group with the near-white flowers of strain C96b (homozygous recessive) and a second group containing all shades of yellow. In each case it is assumed that the homozygous recessive genotype of the main gene (absence of the phenotype) is epistatic over the effects of other genes. We have found markers cosegregating with all three presumptive main loci. The genes *hip* and *via* have been included in the map (Fig. 1). Marker U14.1 cosegregates with the main gene for flower colour (Table 4).

Mapping quantitative traits. While our partial map of the markers is too coarse to determine precise map positions of quantitative trait loci, it adds valuable information for the interpretation of the marker/trait cosegregations. One of the functions of the map is to reduce the number of apparent QTLs by indicating multiple linked markers for one QTL. Table 4 indicates the position of the markers on linkage groups as far as known and shows that significant cosegregations often involve linked markers. A second function is the support by linkage for weak QTL/marker associations. This is illustrated for linkage group g in Fig. 2. Average quantitative values for plants with the dominant and the homozygous recessive marker genotypes are shown and differences between the means significant at the

Table 3. Parental average values (ranges where applicable), average and range of the F₂ plants (based on 200 to 300 F₂ plants depending on character)

Character	A 92	Range	C 96b	Range	F 2	Range
HAIRDAY	135.8	(125–151)	72.6	(69–84)	105.5	(68–147)
BUDDATE	159.5	(154–173)	85.7	(75–98)	122.8	(76–168)
BUD-HAIR	23.7	(9–33)	13.1	(5–19)	16.9	(0–67)
VIOLPET	3.00		0.63		2.04	(0–3)
HIPINT	0		20		11.8	(0–25)
REDPHYL	6		0		1.77	(0–7)
FLVSPHY	3.75		3.60		4.43	(2–8)
FLOWCOL	9		0		6.75	(0–9)
FLSTCOL	10		0		5.55	(0–10)
FLSTLGT	10		7.5		7.43	(0–10)
FLSTINT	5		5		6.48	(0–10)
SCAPLGT	20.2	(16.3–23.0)	29.2	(21.0–37.0)	25.7	(13.4–41.6)
INNPHYL	13.17	(13.0–15.0)	11.01	(8.8–12.3)	13.00	(9.3–18.1)
ACHENES	50.0	(37.8–62.4)	39.0	(32.5–45.2)	50.8	(16.0–90.4)
PCTHAIR	18.51	(15.9–22.0)	11.05	(6.0–15.3)	16.80	(5.5–25.5)
VIAINT	0		9		6.62	(0–10)

5% and the 1% level are indicated. In each case, several linked markers show a significant response. FLSTINT is strongly associated with locus D 5.3. Obviously the weak association with *hip* is a reflection of the same QTL. Since both *hip* and FLSTINT have been scored in 200 plants of the original F₂ population, their association can be confirmed in a much larger statistical sample (Fig. 3).

Similarly, the weak cosegregation of the number of achenes per head with markers U 10.2 and D 5.3 is considerably strengthened by the linkage of these markers (Fig. 2).

Markers U 14.2 and W 9 are linked at a distance of 27.4 cM in a two-point comparison, but were not included in the map due to contradictory associations with other markers. Both markers cosegregate with developmental timing characters (HAIRDATE, BUDDATE; Table 4) and therefore are probably linked.

Pleiotropy. Several of the quantitative characters used in this analysis are partially correlated, for instance HAIRDATE and BUDDATE. Both characters seem to be expressions of the change from vegetative growth to flowering. The linkage of these characters with markers U 14.2 and W 9 indicates a major QTL for developmental timing (vegetative/flowering transition). We have also used the time difference between the BUDDATE and HAIRDATE as a separate quantitative character (BUD-HAIR) in order to detect independent effects on the two component characters that are statistically swamped by the common general timing. This difference cosegregates with a separate marker, U 8.2 (linkage group g). We have found no relationship between BUDDATE and markers on linkage group g, but there are correlations at the 5% probability level between HAIRDATE and markers U 10.2 and *hip*. The latter could be confirmed in a sample of 218 F₂ plants, where the *hip*^o plants (A 92 genotype) had an average hair appearance date of 116.1

Table 4. Co-segregations at a significance level of 1% or better between the two marker genotypes (dominant, homozygous recessive) and a quantitative character. Linkages marked with an asterisk are of inverse polarity (to parental difference or other linkage of same character). QTL# is a tentative identification of 18 independent QTLs underlying these linkages (see text)

Character	Marker	(linkage group)	t	P	QTL#
HAIRDATE	U 14.2		3.29	2.02×10^{-3}	1
	W 9		3.41	1.48×10^{-3}	1
BUDDATE	U 14.2		3.08	3.63×10^{-3}	1
	W 9		3.02	4.33×10^{-3}	1
	p 12A		3.47	2.74×10^{-3}	2
BUD-HAIR	U 8.2	(g)	2.73	$9.31 \times 10^{-3*}$	3
VIOLPET	B 10.2		2.72	9.63×10^{-3}	4
HIPINT	D 3	(d)	3.14	$3.05 \times 10^{-3*}$	5
	<i>hip</i>	(g)	8.20	2.95×10^{-10}	6
	D 5.3	(g)	4.66	3.22×10^{-5}	6
	U 10.2	(g)	3.40	1.55×10^{-3}	6
	C 11		3.00	4.49×10^{-3}	7
REDPHYL	C 2	(d)	2.81	$7.60 \times 10^{-3*}$	8
FLVSPHY	C 4.2	(d)	2.86	6.64×10^{-3}	9
FLOWCOL	U 14.1		9.08	1.82×10^{-11}	10
FLSTCOL	U 14.1		3.78	4.84×10^{-4}	10
FLSTLGT	Y 2.2	(a)	2.87	6.44×10^{-3}	11
	Y 17	(a)	2.84	7.21×10^{-3}	11
	<i>via</i>	(e)	7.74	$1.30 \times 10^{-4*}$	12
	U 17	(e)	5.68	$1.32 \times 10^{-6*}$	12
	U 10.1		3.53	1.09×10^{-3}	13
FLSTINT	A 1.2	(a)	3.07	3.77×10^{-3}	14
	D 5.3	(g)	3.05	3.92×10^{-3}	15
SCAPLGTH	E 17.2	(f)	2.97	$4.97 \times 10^{-3*}$	16
	X 1	(f)	2.79	$8.01 \times 10^{-3*}$	16
INNPHYL	D 5.1		4.01	2.42×10^{-4}	17
ACHENES	D 5.1		3.67	6.79×10^{-4}	17
PCTHAIR	<i>via</i>	(e)	3.84	4.05×10^{-4}	18
	U 17	(e)	3.07	3.81×10^{-3}	18
VIAINT	Y 17	(a)	2.72	$9.91 \times 10^{-3*}$	11
	<i>via</i>	(e)	15.10	1.30×10^{-10}	12
	U 10.1		3.16	3.06×10^{-3}	13

days after planting, while the *hip*⁺ plants (C 96b and heterozygote genotypes) had an average hair appearance date of 106.4 days. The difference is highly significant ($t = 3.603$) and corresponds to 15% of the difference between the parental strains (Table 3).

Another clear pleiotropic effect is the relationship between flower colour and the colour of the stripe on the peripheral florets. The locus contributing to both is the flower colour gene linked with marker U 14.1. The effects of marker D 5.1

Fig. 2. Cosegregation of four phenotypic characters (Table 1) with markers on linkage group (g). Underlined phenotype differences are significant at the 5% level. Thicker lines mark differences significant at the 1% level for BUD-HAIR, HIPINT, and FLSTINT (Table 4)

on the number of inner phyllaries and the number of achenes per head may be pleiotropic consequences of a general increase in capitulum size.

A very intriguing possible case of complex pleiotropy is the relationship between FLSTLGT and VIAINT, two characters expressed only in peripheral florets or the fruit developing from them. Both characters are cosegregating with three unlinked sets of markers, i.e., U 10.1 and markers on linkage groups a and e. We shall analyse the interaction of these loci below.

Multilocus interactions. Taking into account genetic linkages among markers and pleiotropic effects of QTLs, we can tentatively reduce the 32 significant marker/trait associations of Table 4 to the effects of about 18 independent QTL loci (Table 4). This leaves a few quantitative traits (FLSTINT, HIPINT, VIAINT, and FLSTLGT) for which we can demonstrate effects of several relatively strong QTLs. In Tables 5–7 we show the average quantitative trait values associated with the various multimarker genotypes in order to illustrate interactions among QTLs affecting each trait. The notation of the marker genotypes in Tables 5–7 indicates from which of the two parental strains an allele originates and if the marker genotype

Fig. 3. Quantitative segregation for the colour intensity of the violet stripe on the peripheral ligules in 200 F_2 individuals of *Microseris pygmaea* hybrid E 33. Plants with and without trichomes on the inner phyllaries (hip^+ vs. hip^0) marker on linkage group (g) are indicated. This confirms the weak linkage shown in Fig. 2

Table 5. Effect of marker loci D 5.3 and A 1.2 on the transgressive expression of FLSTINT (the colour intensity of the stripe on the outer ligules). Both markers are dominant in parental strain C 96b ("C") and absent in strain A 92 ("a"); genotypes indicate strain origin and dominance of marker alleles

D-5.3	A-1.2	FLSTINT	S.E.	Range
aa	aa	7.0	0.71	6–9
aa	C-	8.2	0.47	5–10
C-	aa	3.9	0.72	1–8
C-	C-	6.6	0.64	1–10

is dominant (present) or homozygous recessive (absent). Thus, marker D 5.3, which is present in strain C 96b ("C") and absent in A 92 ("a") marks the F_2 genotypes "C-" (CC and Ca) and "aa" (Table 5).

Intensity of the floret stripe colour (FLSTINT: Table 5). The intensity of the blue stripe on the peripheral ligules in strain C 96b was judged to be about equal to that of the red stripe in strain A 92, and a score of "5" was given the two parental strains when F_2 plants with much weaker and considerably more intensive colour appeared. Such a transgressive segregation in the F_2 of two homozygous parental strains suggests a minimum of two interacting loci with a weak and a strong allele each. The parental strains are supposed to be reciprocally homozygous for the weak allele of one and the strong of the other locus. We have found strong QTLs near D 5.3 on linkage group g and near A 1.2 on linkage group b with counteracting effects on stripe colour intensity (Table 4). It is obvious from Table 5 that these two loci contribute considerably to the transgressive segregation, but their interaction is probably not simply additive. The QTL-allele linked to (the absence of) D 5.3 in strain A 92 has a strong colour-enhancing effect. When it is homozygous, the stripe colour intensity is always medium or stronger (Table 5: lines 1 and 2),

Table 6. Influence of the main locus *hip* and the marker loci C 11 and D 3 on the density of trichomes on the inner involucrel phyllaries (intensity score HIPINT). Marker genotypes indicate strain of origin (C 96b, A 92) and dominance relationship of each allele. Asterisk marks significant enhancing effect of the C 11-C/D 3-aa genotype on *hip* expression. Expected numbers of F₂ plants are based on Mendelian 3:1 segregations in a sample of 45. One genotype was not found

C 11	D 3	<i>hip</i>	HIPINT	N	N(exp)
aa	aa	aa	0	1	0.7
aa	aa	C-	11	1	2.1
C-	aa	aa	0 (?)	0	2.1
C-	aa	C-	17.69*	13	6.3
aa	C-	aa	0	4	2.1
aa	C-	C-	12.00	6	6.3
C-	C-	aa	0	4	6.3
C-	C-	C-	12.81	16	19.0

Table 7. Influence of the marker loci U 10.1, Y 17, and *via* on the quantitative expression of violet colour on the achenes (intensity score VIAINT; % violet achenes among the light coloured ones) and on the length of the floret stripe (FLSTLGT). Marker genotypes indicate strain of origin (C 96b, A 92) and dominance relationship of each allele. One genotype was not found. Asterisks mark significant (U 10.1- and Y 17-linked) modifier effects on *via*-linked expression

U-10.1	Y 17	<i>via</i>	VIAINT	% viol	FLSTLGT	N
cc	cc	aa	0	0	5.0	1
cc	cc	C-	8.2	52.6	9.8	5
A-	cc	aa	0	0	0.6*	4
A-	cc	C-	8.0	52.7	6.0*	2
cc	A-	aa	not found		not found	—
cc	A-	C-	8.5	56.5	8.4	14
A-	A-	aa	0	0	4.0	2
A-	A-	C-	6.8*	40.3*	8.2	9

and (assuming no crossing over between marker and QTL) a single dose of this allele seems to be sufficient to overcome the colour-reducing effect of the A 92-linked allele near A 1.2, so that a (Ca/aa) combination can determine a colour intensity score of 8 (Table 5: third line).

Density of trichomes on the inner phyllaries (HIPINT: Table 6, Fig. 2). The dominant C 96b allele of the gene *hip* on linkage group g permits the appearance of trichomes on the inner involucrel bracts. Where trichomes may appear, their density varies considerably. Heterozygotes for the main gene tend to have an intermediate density of trichomes. Further loci modify the character so that trichome density on the inner phyllaries in some F₂ plants noticeably exceeds that of the C 96b parent. Two markers affecting trichome density have been found outside of

linkage group g (Table 4). Table 6 shows that the transgressively strong trichome cover on the inner phyllaries of some F₂ plants is associated with a specific 3-locus marker genotype (*hip* C/C, C 11 C/-, D 3 a/a), in which an enhancing effect linked to D 3 on the A 92 genome (only in the presence of a factor from C 96b linked to C 11) explains the transgressive nature of the phenotype.

Even considering the small size of the F₂ selected for mapping, it is striking that the transgressive phenotype was found more often than expected, while the related *hip*[°] (aa) genotype was not found. This raises the possibility that *hip* may not be as all-or-none as we have thought. The “C/-, aa” genotype of the C 11/D 3-linked modifiers that increases the trichome density of *hip*⁺ plants above that of the C 96b parent may induce trichomes even in a homozygous recessive *hip*[°] background. The apparently missing genotype might then be erroneously scored as *hip*⁺ (C/-).

Violet achenes and floret stripe length (VIAINT and FLSTLGT: Table 7). An even more complex pattern of gene interaction concerns the transgressive segregations of the floret stripe length and the violet colour on the peripheral achenes. We have mentioned above that the two characters may be pleiotropically affected by the same interacting gene system (Table 4). Table 7 illustrates the interaction of the various genes in more detail. The columns “VIAINT” and “% viol” are two measures for the same effect: the strength of violet spotting or blotching on the peripheral achenes, determined as a score based on the size and number of violet spots on the fruit wall, and the % among the peripheral (hairy and non-hairy light coloured) achenes that show violet spotting. Both characters are equally affected by the three loci listed in Table 7. The main gene, *via*, determines the presence or absence of violet spots. The A 92-alleles of the two modifiers linked to Y 17 (linkage group a) and U 10.1 act in concert to reduce the strength of violet colour in the peripheral achenes. All three loci therefore cooperate in strain A 92 to prevent the appearance of violet spots on the achenes.

All three loci also affect the length of the coloured stripe on the ligules of the peripheral florets. In all backgrounds, the A 92-allele of *via* or a closely linked factor shortens the stripe. This is remarkable, because both parental strains have long floret stripes, and C 96b rather than A 92 may reduce stripe length due to unfavourable environmental effects. One specific combination of modifiers linked to U 10.1 and Y 17 reduces stripe length even further. In contrast with the effect of these markers on *via* expression, however, a different and recombinant genotype is the active one so that the effect is a transgressive reduction of stripe length (Table 7). The interaction between the two marked regions can be described so that the A 92-allele of a QTL near U 10.1 reduces the expression of *via* in the presence of the A 92-allele of a Y 17 linked QTL, while it reduces the length of the floret stripe in the presence of the C 96b-allele linked to Y 17.

Bud date. The time of appearance of the first capitulum bud is influenced by the locus for general timing (QTL 1 of Table 4 marked by U 14.2 and W 9). In addition, it cosegregates with the three genotypes of the RFLP marker p 12A (QTL 2 of Table 4). The alleles from strain A 92 for both loci delay bud formation. Bud appearance therefore should be directly correlated with the number of A 92 alleles present in the genotype. These have been estimated from the marker combinations (weighed contributions of two dominant markers for one locus plus 0, 1, or 2 alleles from the codominant marker), and the regression of bud appearance time on the

estimated allele number is shown in Fig. 4. The correlation is highly significant ($t = 4.97$, $P = 1.31 \times 10^{-5}$), and the estimated genetic effect explains 36% of the variance in BUDDATE ($R^2 = 0.356$). Each A 92 allele adds on average 14 days (SE 2.83) to the average of 92.5 days (SE 6.59) determined by the C 96b genotype.

Discussion

With proper care, RAPD variation is invaluable for studies where larger numbers of single-locus markers need to be detected quickly and inexpensively. The necessary genetic analysis can be performed relatively easily (ECHT & al. 1992). Typically, RAPD amplification bands are inherited as dominant markers. With additional manipulations they can be converted into codominant markers. However, for a first genetic survey of the genome such as this one for *M. pygmaea*, the simple RAPD method is adequate.

WILLIAMS & al. (1990) were the first to map RAPD loci relative to RFLP variation in a soybean cross. KLEIN-LANKHORST & al. (1991) have identified RAPD markers for the tomato chromosome 6, one of which was closely linked to a nematode resistance gene. REITER & al. (1992) have combined a large number of RAPD loci with RFLPs to obtain a rather dense map of *Arabidopsis thaliana*. UPHOFF & WRICKE (1992) have found RAPD markers cosegregating with a nematode resistance gene in sugar beet. In order to find RAPD markers for specific genes, differences in the amplification pattern between the pooled DNAs of all F_2 plants containing one or the other genotype for the gene of interest have been used (PARAN & al. 1991, MARTIN & al. 1991, MICHELMORE & al. 1991).

We have here used RAPD markers to find genes involved in the phenotypic evolution of a newly arisen species. DOEBLEY & al. (1990) have used the segregation of RFLPs in a maize-teosinte F_2 population to detect genes involved in the evolution of maize. The genetic differences in some natural progenitor-derivative species pairs have previously been analysed using isoenzymes (reviewed in PLEASANTS & WENDEL 1989) and quantitative genetic methods (MACNAIR & CUMBES 1989). Two related questions have been addressed in these studies. One concerns various ideas about the genetic processes involved in speciation (COYNE & BARTON 1988), the other concerns the genetic basis of morphological differences among species. In both cases, the literature reflects an apparent polarization of views. Speciation is either

Fig. 4. Regression of the appearance of the first capitulum bud (day after planting) on the estimated number of alleles from parent A 92 at two loci (marked by U 14.2/W 9 and p 12A)

seen as a by-product of adaptive differentiation and involves selection in quantitative multigenic systems (BARTON & CHARLESWORTH 1984, COYNE & LANDE 1985) or speciation is considered the outcome of a revolutionary restructuring of the genome in a population during a temporary reduction in numbers (CARSON & TEMPLETON 1984), and morphological differentiation between species as the result a few changes in genes with large effects (HILU 1983, MARX 1983, GOTTLIEB 1984).

Microseris pygmaea is the result of chance long-distance dispersal. If anything, it demonstrates that there are many ways in which plant species can arise. We still have insufficient information about the structure of the ancestral North American genome. The mutation leading to a duplication of pappus part numbers is the only one clearly involved in the speciation event. Genetic variation at this locus is not expressed in the phenotype of the intraspecific hybrid which we have analysed here. There is little evidence for a “genetic revolution” in the origin of *M. pygmaea*. Originally, one specimen from a North American population may have been transported to Chile and landed on a spot suitable for its germination, survival and reproduction. The phylogenetic analysis shows that most genetic changes in the species have occurred later, and we do not even know yet which of these changes are adaptive and which are the result of drift during subsequent founder events. Still, considering the striking rarity of species-specific characters in *Microseris*, finding at least one very conspicuous character change associated with the speciation event is noteworthy.

We are getting an ever more detailed picture of the genetics of the phenotypic differences that have accumulated within the species. The genomes combined into strain E 33 represent the extremes of the two independent lines of intraspecific evolution. Since we have a tentative cladogram of the evolutionary steps connecting these extreme populations via their common ancestor (BACHMANN & al. 1985 a, VAN HEUSDEN 1990, VAN HEUSDEN & BACHMANN 1992), we hope eventually to assign the various allelic differences to specific nodes in the cladogram. The two monophyletic subgroups of populations corresponding to coastal and inland strains, for instance, differ in flowering time, the number of outer involucre bracts and the percentage of hairy peripheral achenes. The coastal strains, including C 96, have about 11% hairy achenes, while the inland strains, including A 92, have about 18% (one inland population is polymorphic for this and other characters; BACHMANN & al. 1985 b). We have found one major QTL for this difference in hybrid E 33 (Table 4), which may mark one of the diagnostic mutations differentiating coastal and inland strains. The linkage of this gene with *via* and U 17 may eventually help to decide which of the two character states (if any) is the ancestral, “Californian” one. The closely linked mutation of *via* leading to the absence of violet colour on the peripheral achenes is an independent, later evolutionary event which occurred in the common ancestor of the advanced inland populations (VAN HEUSDEN & BACHMANN 1992).

The variation found here includes alternative alleles fixed in different populations and polymorphisms in individual populations. The blue stripe on the outer florets is found only in one (i.e., C 96b) of two biotypes coexisting (as homozygotes) in the coastal population C 96. The stripe is red in the other biotype (C 96r) and most other populations of *M. pygmaea*, and there is a distinct reduction in its intensity in the most extreme inland strains including A 92 (BACHMANN & al. 1985 b). It is

therefore not too surprising that at least 7 loci affecting stripe colour, length, and colour intensity segregate in E 33 (QTLs 9 to 14 of Table 4 plus at least one unlinked minor effect). There is also considerable variation for capitulum size among the populations. The one major QTL for this character (ACHENES in Table 3) is only one of 5 detected at a less stringent significance level of 5% (see Fig. 2). As with the loci affecting the floret stripe, various loci affecting the number of achenes per head have different polarity of action. The transgressive segregations found for several characters indicate that along the phylogenetic path connecting A 92 and C 96 via their common ancestor, mutations with opposite phenotypic effects in loci affecting these characters have become fixed. There is no indication of a sustained trend in the evolution of these characters within the species. Other authors have found QTL alleles with effects opposite to those found in the parental strains from which they have been derived (TANKSLEY & al. 1982, 1988). In *M. pygmaea* we can see some of the phylogenetic intermediate steps by which these have arisen.

The availability of linked markers makes it possible to estimate gene numbers and gene effects (EDWARDS & al. 1987). A hybrid segregating for markers distributed more or less equally over all chromosomes should permit a relatively complete count of QTL loci (PATERSON & al. 1988, DOEBLEY & al. 1990, KEIM & al. 1990). If the strains that have been crossed represent evolutionary landmarks such as a progenitor and a derivative species (DOEBLEY & al. 1990) or the extremes of the intraspecific differentiation as in *M. pygmaea*, counting and mapping the QTL loci responsible for their genetic distance and identifying their effects, will eventually provide the ultimate level of resolution in evolutionary genetics. Our calculations of the phenotypic effects due to the multilocus genotypes of marked QTLs have shown that our data are still incomplete. There are more, and smaller, effects to be taken into account for a reasonably complete analysis of the difference between the two strains.

The number of genes affecting a character depends to a certain extent on the intensity of sampling and mapping. There are at the moment two approaches for identifying QTLs relative to a marker map, one using single markers, one based on the relationship of linked markers. STUBER & al. (1993) have compared these approaches in maize using a complete map containing 76 appropriately spaced RFLPs and have obtained virtually the same results with either method. With only part of our markers on an incomplete map, we have used single-marker associations to find QTLs. Also, our relatively small sample of plants limits the detection of QTLs associated with the available markers. In this respect, the detection of a marker associated with the difference between the timing characters HAIRDATE and BUDDATE but not (significantly) with either of the separate characters shows that both marker number and density and the number of plants in the sample eventually need to be increased. As the statistics for the detection of QTLs get refined, some of the many marginal effects found here (those not listed in Table 4) may turn out to be sampling errors, others will be confirmed as true QTLs, and more QTLs with minor effects will become visible. Especially there will be more pleiotropic effects of genes determining basic features such as flowering time and capitulum size. The difference between single genes interacting in the determination of a quantitative character and a residual multigenic "genetic background" may be a technical rather than a biological one.

We have found a few characters that show the inheritance pattern of a major gene with multiple modifiers (e.g., *hip*, *via*). In spite of the essentially qualitative effect of these main genes, they influence characters of probably minor adaptive importance. This is different in other cases. DOEBLEY & al. (1990) found single loci with major effects for four essential character differences between maize and teosinte. In *Microseris*, we have found at least two characters in which one major gene effect was discovered in crosses between species, while the same character segregated in a quantitative polygenic pattern in intraspecific crosses (VLOT & al. 1992). In these cases we have the impression that the major gene effect occurred during speciation, may even have been part of the speciation event, and that the intraspecific polygenes are modifiers which have been selected to stabilize and modify the original gene effect. The pappus part system of *M. pygmaea* may be a further case with the added feature that intraspecific genetic variation for the character is not even expressed in the phenotype. The importance of resolving superficially quantitative multigenic systems has been stressed by BACHMANN (1983: 203). Since then the necessary molecular marker techniques have become available, and we can expect more detailed analyses of multigenic systems and their evolutionary origin.

References

- BACHMANN, K., 1983: Evolutionary genetics and the genetic control of morphogenesis in flowering plants. — *Evol. Biol.* **16**: 157–208.
- 1991: Genetic variation for meristic characters of the capitula of *Microseris pygmaea* (*Asteraceae: Lactuceae*). — *Biol. Zentralblatt* **110**: 145–156.
- VAN HEUSDEN, A. W., CHAMBERS, K. L., PRICE, H. J., 1985 a: Duplications of additively acting genes in the evolution of a plant (*Microseris pygmaea*). — *Experientia* **41**: 1348–1350.
- CHAMBERS, K. L., GRAU, J., PRICE, H. J., 1985 b: Genetic variation in *Microseris pygmaea* (*Asteraceae-Lactuceae*). — *Beitr. Biol. Pflanzen* **60**: 51–88.
- BARTON, N. H., CHARLESWORTH, B., 1984: Genetic revolutions, founder effects and speciation. — *Annu. Rev. Ecol. Syst.* **15**: 133–164.
- BATTJES, J., BACHMANN, K., BOUMAN, F., 1992: Early development of capitula in *Microseris pygmaea* D. DON strains C96 and A92 (*Asteraceae; Lactuceae*). — *Bot. Jahrb. Syst.* **113**: 461–475.
- CARSON, H. L., TEMPLETON, A. R., 1984: Genetic revolutions in relation to speciation phenomena: the founding of new populations. — *Annu. Rev. Ecol. Syst.* **15**: 97–131.
- CHAMBERS, K. L., 1955: A biosystematic study of the annual species of *Microseris*. — *Contrib. Dudley Herb. Stanford* **4**: 207–312.
- 1963: Amphitropical species pairs in *Microseris* and *Agoseris* (*Compositae: Cichorieae*). — *Quart. Rev. Biol.* **38**: 124–140.
- COYNE, J. A., BARTON, N. H., 1988: What do we know about speciation? — *Nature* **331**: 485–486.
- LANDE, R., 1985: The genetic basis of species differences in plants. — *Amer. Naturalist* **126**: 141–145.
- DELLAPORTA, S. L., WOOD, J., HICKS, J. B., 1983: A plant DNA miniprep version II. — *Pl. Mol. Biol. Rep.* **1**: 19–21.
- DOEBLEY, J., STEC, A., WENDEL, J., EDWARDS, M., 1990: Genetic and morphological analysis of a maize-teosinte F₂ population: implications for the origin of maize. — *Proc. Natl. Acad. Sci. USA* **87**: 9888–9892.

- ECHT, C. S., ERDAHL, L. A., MCCOY, T. J., 1992: Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. — *Genome* **35**: 84–87.
- EDWARDS, M. D., STUBER, C. W., WENDEL, J. F., 1987: Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution, and types of gene action. — *Genetics* **116**: 113–125.
- GOTTLIEB, L. D., 1984: Genetics and morphological evolution in plants. — *Amer. Naturalist* **123**: 681–709.
- HILU, K. W., 1983: The role of single gene mutations in the evolution of flowering plants. — *Evol. Biol.* **16**: 97–128.
- KEIM, P., DIERS, B. W., OLSON, T. C., SHOEMAKER, R. C., 1990: RFLP mapping in soybean: association between marker loci and variation in quantitative traits. — *Genetics* **126**: 735–742.
- KLEIN-LANKHORST, R. M., VERMUNT, A., WEIDE, R., LIHARSKA, T., ZABEL, P., 1991: Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). — *Theor. Appl. Genet.* **83**: 108–114.
- MACNAIR, M. R., CUMBES, Q. J., 1989: The genetic architecture of interspecific variation in *Mimulus*. — *Genetics* **122**: 211–222.
- MARTIN, G. B., WILLIAMS, J. G. K., TANKSLEY, S. D., 1991: Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. — *Proc. Natl. Acad. Sci. USA* **88**: 2336–2340.
- MARX, G. A., 1983: Developmental mutants in some annual seed plants. — *Annu. Rev. Pl. Physiol.* **34**: 389–417.
- MICHELMORE, R. W., PARAN, I., KESSELI, R. V., 1991: Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. — *Proc. Natl. Acad. Sci. USA* **88**: 9828–9832.
- PARAN, I., KESSELI, R., MICHELMORE, R., 1991: Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near-isogenic lines. — *Genome* **34**: 1021–1027.
- PATERSON, A. H., LANDER, E. S., HEWITT, J. D., PETERSON, S., LINCOLN, S. E., TANKSLEY, S. D., 1988: Resolution of quantitative traits into mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. — *Nature* **335**: 721–726.
- PLEASANTS, J. M., WENDEL, J. F., 1989: Genetic diversity in a clonal narrow endemic, *Erythronium propullans*, and its widespread progenitor, *Erythronium albidum*. — *Amer. J. Bot.* **76**: 1136–1151.
- REITER, R. S., WILLIAMS, J. G. K., FELDMANN, K. A., RAFALSKI, J. A., TINGEY, S. V., SCOLNIK, P. A., 1992: Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNA. — *Proc. Natl. Acad. Sci. USA* **89**: 1477–1481.
- STAM, P., 1993: Construction of integrated genetic linkage maps by means of a new computer package: JOINMAP. — *Pl. J.* (In press.)
- STUBER, C. W., LINCOLN, S. E., WOLFF, D. W., HELENTJARIS, T., LANDER, E. S., 1993: Identification of genetic factors contributing to heterosis in a hybrid from two Elite maize inbred lines using molecular markers. — *Genetics* **132**: 823–839.
- TANKSLEY, S. D., HEWITT, J., 1988: Use of molecular markers in breeding for soluble solids content in tomato—a re-examination. — *Theor. Appl. Genet.* **75**: 811–823.
- MEDINA-FILHO, H., RICK, C. M., 1982: Use of controlling quantitative traits in an interspecific backcross of tomato. — *Heredity* **49**: 11–25.
- UPHOFF, H., WRICKE, G., 1992: Random amplified polymorphic DNA (RAPD) markers in sugar beet (*Beta vulgaris* L.): mapping the genes for nematode resistance and hypocotyl colour. — *Pl. Breeding* **109**: 168–171.

- VAN HEUSDEN, A. W., 1990: Genetic analysis of natural variation in *Microseris pygmaea* (Asteraceae) in crosses with *M. bigelovii* C 93b. — PhD Thesis, University of Amsterdam.
- BACHMANN, K., 1992: Genetic differentiation of *Microseris pygmaea* (Asteraceae, Lactuceae) studied with DNA amplification from arbitrary primers (RAPDs). — Acta. Bot. Neerl. **41**: 385–395.
- — CHAMBERS, K. L., 1989: Variation in time and place of trichome appearance in *Microseris* hybrid J 05 (*M. pygmaea* × *M. bigelovii*, Asteraceae). — Biol. Zentralbl. **108**: 153–161.
- VAN HOUTEN, W. H. J., VAN HEUSDEN, A. W., ROUPPE V. D. VOORT, J., RAYMANN, L., BACHMANN, K., 1991: Hypervariable DNA fingerprint loci in *Microseris pygmaea* (Asteraceae, Lactuceae). — Bot. Acta **104**: 252–256.
- VLOT, E. C., VAN HOUTEN, W. H. J., MAUTHE, S., BACHMANN, K., 1992: Genetic and non-genetic factors influencing deviations from five pappus parts in a hybrid between *Microseris douglasii* and *M. bigelovii* (Asteraceae, Lactuceae). — Int. J. Pl. Sci. **53**: 89–97.
- WILLIAMS, J. G. K., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A., TINGEY, S. V., 1990: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. — Nucl. Acid Res. **18**: 6531–6535.

Addresses of the authors: K. BACHMANN (correspondence), W. H. J. VAN HOUTEN, Hugo de Vries Laboratory, University of Amsterdam, Kruislaan 318, NL-1098 SM Amsterdam, The Netherlands. — L. W. D. VAN RAAMSDONK, Centrum voor Plantenveredeling en Reproductieonderzoek CPRO, Postbus 16, NL-6700 AA Wageningen, The Netherlands.

Accepted November 16, 1993 by R. EHRENDORFER