Org. Divers. Evol. 2, 277–288 (2002)

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ORGANISMS DIVERSITY & EVOLUTION

QTL analysis reveals different and independent modes of inheritance for diagnostic achene characters in *Microseris* (Asteraceae)

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Received 22 February 2002 · Accepted 6 June 2002

Abstract

There are few reliable diagnostic morphological characters for species of the asteracean genus *Microseris,* and quantitative differences in the shapes of the achenes and the paleaceous pappus parts play a decisive role in species recognition. The genetic basis of species and strain differences in various characters has been studied previously, but little is known about quantitative characters of the achenes.
We performed a quantitative trait locus (QTL) analysis in the F2 of an interspecific cross between *Microseris douglasii* and *M. bigelovii* for achene length, achene diameter, achene shape, palea length, awn length, and achene pigmentation.
Independent inheritance of the main heritable achene characters – achene length, palea length and achene pigmentation – was revealed by the detection of distinct and specific QTLs for these characters. For palea length five QTLs with about equal phenotypic effects were mapped on four different linkage groups. Achene length and achene shape (achene length / achene diameter) were determined by two different genetic systems with one major gene and two modifiers. The detection of QTLs with a polarity of the effects opposite to that in the parental strains for achene length and achene shape reveals genetic variation for a potential increase in species differences. For the highly heritable trait, achene pigmentation, the bimodal F2 distribution suggested single-factor inheritance for absence versus presence of spots, with dominance for the spotted condition. However, only relatively weak QTL effects on that trait could be detected. Additional molecular markers (RAPDs, AFLPs) have to be tested for cosegregation with that major gene. The results are discussed in the context of different theories for the evolution of morphological characters.

Key words: diagnostic characters, achenes, *Microseris*, Asteraceae, RAPD, QTL

Introduction

The genetics of morphological differentiation among natural plant species is still poorly understood, although there is a growing number of QTL studies indicating a range of contrasting modes of inheritance. Many of those QTL studies found one or a few major genes for quantitative and for diagnostic (qualitative) character differences (Vlot et al. 1992, Hombergen & Bachmann 1995, Lin & Ritland 1997, Bradshaw et al. 1998, Hill & Doebley 1999, Gailing et al. 1999, Lauter & Doebley 2002), contrary to the classical view that always many minor and unlinked genes control those character differences (Fisher 1930, Dobzhansky 1937, Coyne & Lande 1985, Levin 2001). Molecular phylogenetic studies have shown that considerable morphological differences can occur in closely related monophyletic groups, indicating simple genetic differences with major phenotypic effects (Sytsma & Gottlieb 1986a, b; Sytsma 1990; Kadereit 1994). Those results shed new light on the ongoing debate, whether the evolution of morphological character differences is the result of change in a few major genes (Bachmann 1983, Hilu 1983, Gottlieb 1984) or of gradual change of many loci with small effects (Coyne & Lande 1985, Orr & Coyne 1992).

We are using the annual species of the genus *Microseris* D. Don (Asteraceae: Lactuceae) as a model system for an in-depth analysis of the genetic basis of taxonomically useful characteristics. With the exception of the Chilean *M. pygmaea* D. Don, the three diploid and

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two allotetraploid Californian species included in Section *Microseris* (Chambers 1955) form a taxonomically 'difficult group' for various reasons: there are many populations containing more than one of these species, and since all of the species are reproducing primarily by selfing, there is much intraspecific variation in all characters, even in those that are used for species identification (Chambers 1955). There is not much evidence for interspecific hybridization, but it must have occurred occasionally, since *M. campestris* Greene was shown to be a tetraploid hybrid between *M. douglasii* (D.C.) Sch.-Bip. and *M. elegans* Greene ex Gray (Chambers 1955, Roelofs et al. 1997). Hybridization with *M. bigelovii* (Gray) Sch.-Bip. has led to chloroplast introgression into *M. douglasii* with no demonstrable introgression of nuclear genes (Roelofs & Bachmann 1997a).

The only character that showed a non-random geographic distribution within the general distribution area of the widespread *M. douglasii* was the color intensity (not the variable color itself) of the stripe on the abaxial surface of the outer florets of the capitula (Bachmann & Battjes 1994). The taxonomic treatment of the group relies strongly on the morphology of the ripe achenes (Fig. 1; Chambers 1955: figs 13 and 14, Irmler et al. 1982). The differences in achene morphology between *M. douglasii* subsp. *douglasii* and *M. bigelovii* are illustrated in Fig. 1. 'Plastic responses' to non-optimal environmental conditions tend to reduce primarily the number of achenes while keeping achene size and shape more or less constant (Bachmann & Roelofs 1995). Chambers (1955: figs 11 and 12) has looked at the variation of fruit morphology in natural populations and in offspring families of plants in order to obtain an impression of the genetics of the characters. Since then additional genetic data have been obtained on achene characters, especially on variation in pappus part number (Chambers 1963, Bachmann & Chambers 1978, Bachmann et al. 1981, 1982, 1984, Zentgraf et al. 1984, Mauthe et al. 1985, Bachmann et al. 1985, Vlot & Bachmann 1991, Vlot et al. 1992).

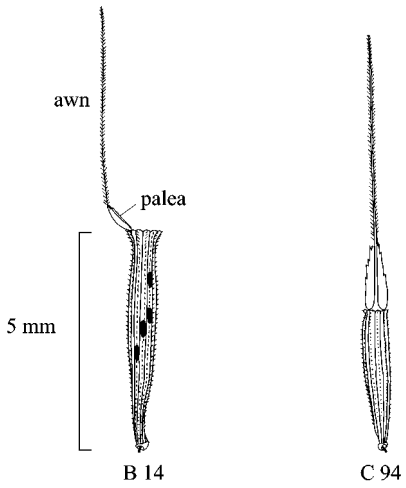

Fig. 1. Typical achenes of *Microseris douglasii* (B14) and *Microseris bigelovii* (C94), modified from Chambers (1955).

With the advent of molecular marker techniques, it has become possible to map genes affecting quantitative characters as "quantitative trait loci" (QTLs) relative to a genetic marker map. This has been applied to achene spotting in *M. pygmaea* (Van Houten et al. 1994) and to pappus part number in an interspecific cross between *M. douglasii* and *M. bigelovii* (Vlot et al. 1992, Bachmann & Hombergen 1996).

Molecular analysis has confirmed the common derived position of the three annual species *M. bigelovii*, *M. elegans* and *M. pygmaea* relative to *M. douglasii* (Wallace & Jansen 1995; Roelofs & Bachmann 1997a, b; Roelofs et al. 1997). Here, we want to use the same cross between *M. douglasii* and *M. bigelovii* to examine those characters of the shape of achenes and of the pappus parts (paleae) that were used by Chambers (1955) as taxonomically relevant characters to distinguish the parental species of our mapping population.

Material and methods

Plant material

The F2 hybrid population (H27-2) was derived from an interspecific cross between *M. douglasii* (strain B14) and *M. bigelovii* (strain C94). *M. douglasii* B14 (Chambers accession number CH4284) was collected near Parkfield, Fresno County, CA, by K. L. Chambers on 3 April 1977, *M. bigelovii* C94 (CH-A303) was collected in Uplands Park, Victoria, B.C., Canada, by M. C. Melburn on 28 May 1967. One head of inbred strain B14 was crossed with C94 as pollen donor by J. Battjes in 1991. An F2 population of 106 plants was obtained from one hybrid plant (H27) that was raised in fall 1992 together with parental controls. In spring 1993 achenes were harvested from the F2. F3 plants were raised in 1993/1994, and achenes were harvested in spring 1994.

Scoring procedures

We scored the following achene characteristics: length of the achene, diameter of the achene at its widest part, length of the palea, length of the awn, and achene spotting (Fig. 1).

Measurements of achene characters were performed with a stereo microscope (Stemi SV 6; Zeiss) at 25× (length parameters) and 50× (achene diameter) magnification, respectively, in a segregating population of 89 F2 plants and 411 F3 plants. Achene shape as a composite character was calculated as the quotient of achene length and achene diameter (AL/AD) (Table 1). Achene pigmentation was scored on a scale from 0 (no spots) to 4 (strongly spotted).

From each F2 and F3 line, ten achenes were measured and the mean values calculated. For the test of trait heritability the mean of the five F3 lines derived from one F2 plant was regressed on corresponding F2 values. A total of one hundred achenes were scored for achene characters in ten offspring plants of each of our crossing parents B14 and C94.

Data analysis

The molecular marker map was calculated on the basis of the segregation of 289 random amplified polymorphic DNA markers (RAPDs) in the 106 F2 plants of the interspecific hybrid (H27) between *M. douglasii* and *M. bigelovii* (Hombergen & Bachmann 1995), using the computer program JOINMAP (Stam 1993). 214 of those 289 polymorphic markers could be assigned to 16 linkage groups (Bachmann & Hombergen 1996). A detailed description of this linkage map and QTLs for other phenotypic traits are given elsewhere (Hombergen & Bachmann 1995, Bachmann & Hombergen 1996). QTL analysis was performed with Qgene (Nelson 1997) using simple linear regression (Haley & Knott 1992) and interval analysis (Lander & Botstein 1989). The coefficient of determination, R^2, was used as an estimate for the phenotypic variance explained (PVE). QTL genotypes for each line were inferred from linked markers with opposite polarity (from one parent or the other), and a linear regression of the phenotypic values was performed for each trait against zero, one or two alleles of one parent (Bachmann & Hombergen 1996). The number of QTLs for one character and their contribution to the total phenotypic variance explained (PVE_{total}) were estimated by multiple regression analysis (Nelson 1997). The coefficient of determination (R^2) of the multiple regression analysis was used as an estimate of PVE_{total}. Potential epistatic interactions between different alleles of trait-specific QTLs were tested by linear regression of all QTLs on the homozygous *M. douglasii* and *M. bigelovii* genotypes of all other QTLs.

Results

All traits concerning quantitative achene characters are normally distributed (Fig. 2). The achenes of *M. douglasii* are generally longer and have a higher achene shape quotient (achene length/achene diameter) than the *M. bigelovii* achenes, whereas the palea of *M. bigelovii* is more than twice as long as that of *M. douglasii* (Fig. 1). For achene length, achene diameter and awn length, the *M. douglasii* parent shows much more phenotypic variation than the *M. bigelovii* parent, whereas for achene shape and palea length phenotypic ranges of the parents are more distinct and of about the same width (Fig. 2). Except for awn length and achene diameter, phenotypic ranges of the crossing parents are not overlapping (Fig. 2). Achenes of the parent *M. bigelovii* are unspotted, whereas those of *M. douglasii* are always covered with violet spots in different strength (Fig. 1). This trait shows a bimodal distribution of spotted versus unspotted achenes in the F2 that is in accordance with the segregation of one major gene with dominance for the spotted condition ($\chi^2 = 1.98$; $0.10 < p < 0.20$). The strength of pigmentation of the spotted achenes is continuously distributed. No correlation between the traits achene length, achene diameter, palea length and achene pigmentation could be detected in the F2 of 106 plants (data not shown). The heritability (determined as the coefficient of determination, R^2) under our greenhouse conditions of all traits except achene diameter is more than 50% (Table 1). Regarding absence versus presence of spots on achenes, all F3 offspring of unspotted F2 plants are unspotted, and all five F3 offspring of spotted F2 plants either are all spotted or show segregation in spotted and unspotted achenes.

QTL analysis

For each QTL, markers with the most profound phenotypic effect and their respective map positions are listed in Table 2. The peaks of these QTLs found by linear regression correspond to those found by interval mapping (interval size 0.5 cM). For QTLs where heterozygotes could be inferred, mostly additive inheritance (d/a = 0)

Table 1. Trait description of the measured achene characters. Mean values and phenotypic ranges for metric characters (in mm) and for achene pigmentation are given for ten plants of each crossing parent (C94 and B14) and for the 89 F2 and 411 F3 plants. For each plant ten achenes were scored. Achene pigmentation was scored on a scale from 0 (no spots) to 4 (strongly spotted).

Trait	Trait description	Heritability (%); R^2	F2 mean	F2 range	F3 mean	F3 range	C94 mean	C94 range	B14 mean	B14 range
AL	achene length	69.5	4.44	3.31–5.58	4.33	3.02–6.02	3.52	3.37–3.73	5.08	4.36–5.64
AD	achene diameter	32.9	0.79	0.53–0.93	0.76	0.53–0.99	0.77	0.75–0.78	0.80	0.65–0.89
AS (= AL/AD)	achene shape	68.1	5.70	3.99–10.11	5.75	3.68–10.02	4.57	4.38–4.78	6.41	6.00–6.75
PAL	palea length	58.5	1.88	0.33–3.22	1.60	0.23–4.72	2.67	2.52–2.88	0.96	0.78–1.26
AW	awn length	50.1	5.17	4.02–6.12	4.87	3.12–6.60	4.87	4.78–5.08	5.05	4.54–5.26
SPOT	achene pigmentation	72.8	1.55	0–3.75	1.23	0–4.00	0		2.67	1.90–3.00

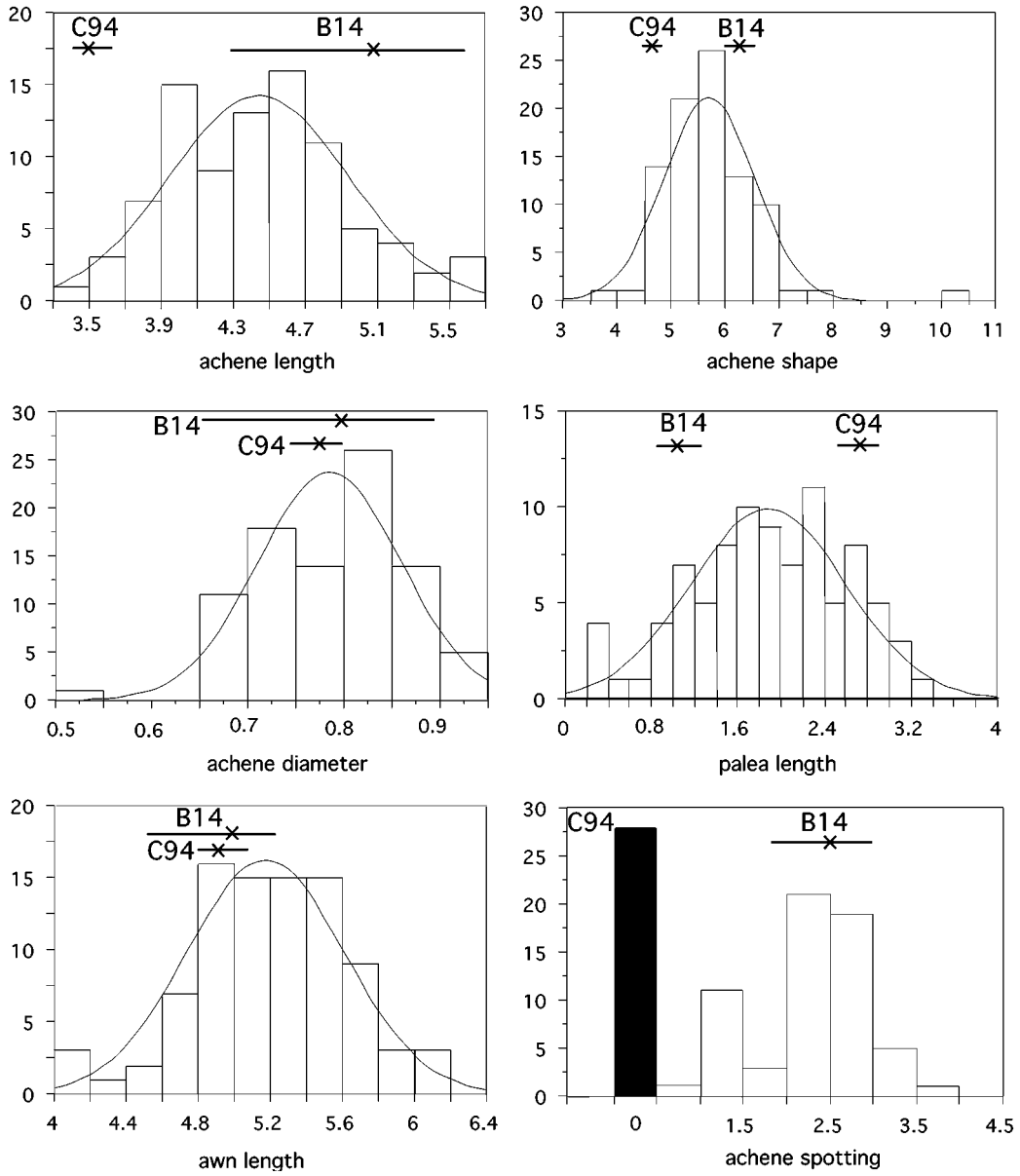

Fig. 2. Frequency distributions of achene characters in the F2 of an interspecific cross between *Microseris douglasii* (B14) and *Microseris bigelovii* (C94). Phenotypic ranges and mean values measured in one hundred achenes of the parental controls are shown as horizontal bars and crosses, respectively. For the character achene spotting, all lines in the black bar and all C94 plants have unspotted achenes.

could be shown for the metric achene characters achene length, achene shape, palea length, and awn length (Table 2). In QTLs PAL08R (d/a = 0.292), AW08R (d/a = 0.324), and SPOT03R (d/a = 0.357), the *M. douglasii* allele shows partial dominance. The phenotypic variance explained (PVE) by each QTL (R^2 of linear regression) and the total PVE_{total} (R^2_{total} of multiple regression) for each trait are summarized in Table 2.

For achene length one major QTL on linkage group 02R (AL02R, $R^2 = 0.275$) and two minor QTLs on linkage group 03R (AL03R, $R^2 = 0.130$) and on linkage group 04R (AL04R, $R^2 = 0.146$) were confirmed by linear and multiple regression (Table 2, Fig. 3).

The same markers on linkage group 02R that showed the strongest marker phenotype correlation with achene length also showed significant correlation with achene

Table 2. Quantitative trait loci (QTLs) for achene characters. d/a = degree of dominance. The direction of dominance is indicated in brackets. R^2_{total} is a measure for PVE_{total} = total phenotypic variance explained. For trait descriptions and measures see Table 1.

	Regression analysis					QTL phenotypes					R^2_{total}		Interval analysis		
	QTL markers	Position (cM)	QTL name	R^2	p-value	B14/B14	B14/C94	C94/C94	Mean diff.	d/a		p-value	Lod-score	p-value	Position (cM)
AL	OPX03.06 (C94)	68.4	AL02R	0.275	<0.0001	4.75	4.40	4.00	0.75	0.070 (doug)	0.379	<0.0001	6.64	<0.0001	67
	OPA01.01 (B14)	71.1													
	OPD15.01 (C94)	76	AL04R	0.146	<0.0001	4.07		4.52	0.45				4.32	0.0001	65.5
	OPF04.06 (C94)	37.2	AL03R	0.130	<0.0001	4.72		4.32	0.40				3.60	0.0003	38
AD	OPA10.05 (C94)	76.9	AD02R	0.122	0.0004	0.733		0.795	0.062		0.207	<0.0001	3.29	0.0006	76.5
	OPA18.02 (B14)	27.9	AD06R	0.069	0.0086	0.812		0.743	0.069				2.1	0.0092	27
AS	OPW07.02 (C94)	72.7	AS02R	0.266	<0.0001	6.38	5.58	5.08	1.30	–0.232 (big)	0.331	<0.0001	8.02	<0.0001	75
	OPA12.06 (B14)	78.2													
	OPF11.02 (C94)	22.9	AS03R	0.134	0.0002	6.24		5.53	0.71				3.52	0.0004	22
	OPA18.02 (B14)	27.9	AS06R	0.091	0.0024	5.57		6.15	0.58				2.75	<0.0001	32
PAL	OPC07.04 (B14)	20.8	PAL01aR	0.154	<0.0001	1.66		2.37	0.71		0.450	<0.0001	6.63	<0.0001	20
	OPB19.03 (B14)	64.1	PAL01bR	0.148	0.0002	1.48	1.78	2.27	0.79	0.240 (doug)			3.14	0.0009	65.5
	OPH05.02 (C94)	68.3													
	OPY04.02 (C94)	16.2	PAL04R	0.145	0.0001	1.33		1.97	0.64				4.67	<0.0001	14
	OPC14.02 (B14)	68.3	PAL03R	0.135	0.0003	1.35	1.77	2.12	0.77	–0.088 (big)			3.13	0.0009	69.5
	OPF12.03 (C94)	72.9													
	OPF10.07 (B14)	31.4	PAL08R	0.108	0.0090	1.57	1.80	2.23	0.66	0.292 (doug)			2.77	0.0021	31
	OPA20.02 (C94)	52.5													
AW	OPF10.07 (B14)	31.4	AW08R	0.083	0.0040	5.30	5.16	4.88	0.42	0.324 (doug)			2.54	0.0034	33
	OPD05.02 (C94)	0													
SPOT	OPB04.01 (B14)	31	SPOT13R	0.141	0.0001	1.78		0.79	0.99		0.280	<0.0001	3.29	0.0006	28
	OPC14.02(B14)	68.3	SPOT03R	0.133	0.0004	2.04	1.67	0.89	1.15	0.357 (doug)			2.78	0.0020	70
	OPF12.03 (C94)	72.9													

shape. By testing the effect of this major achene length QTL (AL02R) on achene shape (R^2 = 0.169) and achene diameter (R^2 = 0.001) it could be shown that AL02R influences the composite variable achene shape only via achene length (R^2 = 0.275) (Table 3). The major achene shape QTL (AS02R) (R^2 = 0.266) is also located on linkage group 02R close to AL02R. This QTL has significant effects on achene length (R^2 = 0.197) and on achene diameter (R^2 = 0.050) (Table 3). However, AS02R showed no 'synergistic' effects with AL02R on achene length (tested by multiple regression analysis). Two modifiers for achene shape are located on linkage group 03R (AS03R; R^2 = 0.134) and on linkage group 06R (AS06R; R^2 = 0.091).

Only single marker signals could be detected for achene diameter on linkage group 02R, overlapping with the major achene shape QTL AS02R (AD02R), and on linkage group 06R (AD06R = AS06R). AD06R showed cosegregation specifically with achene shape (R^2 = 0.091) and achene diameter (R^2 = 0.069), but no significant effect on achene length (R^2 = 0.021, p = 0.1553) (Table 3).

In two QTLs, effects in the opposite direction of the species differences could be observed. This is the case in one achene length QTL (AL04R; marker OPD15.01) and in one achene shape QTL (AS06R = AD06R; marker OPA18.02) where the allele from the *M. bigelovii* parent (with short and stout achenes) is associated with long and oblong (= high AL/AD value) achenes, respectively.

The pappus is composed of the palea and the awn (Fig. 1). Even though the awn is normally more than twice as long as the palea, most of the variation in pappus length is derived from variation in palea length (r = 0.852 for palea length, r = 0.507 for awn length). QTL analysis confirmed that variation in pappus length is mainly due to genetically determined variation in palea length, whereas for awn length only one significant signal on linkage group 08R (AW08R) could be found. Marker OPF 10.07 from *M. douglasii* on that linkage group has significant opposite effects (p < 0.005) on palea length and awn length variation. The *M. douglasii* allele is associated with a shorter palea and with a longer awn. The composite character pappus length (= awn length plus palea length) is not affected by this QTL (Table 2, Fig. 2).

In total, five QTLs with about equal – mainly additive – effects on the phenotype could be mapped on four linkage groups for palea length (Table 2, Fig. 3). Only QTL PAL08R shows partial dominance for the *M. douglasii* allele (see above). Multiple regression analysis revealed the existence of two individual QTLs for palea length on linkage group 01R with strongly synergistic effects (PAL01aR, R^2 = 0.154; PAL01bR, R^2 = 0.148; $R^2_{multiple}$ = 0.288). In all of those five QTLs the *M. bigelovii* genotype is associated with the higher phenotypic mean.

Independent inheritance of achene characters was confirmed by the location of the strongest QTLs for the main heritable metric achene components, achene length / achene shape (lg02R) and palea length (lg01R), on different linkage groups (Fig. 3).

A high amount of the total phenotypic variance is explained for achene length (PVE_{total} 37.9%, heritability 69.5%), achene shape (PVE_{total} 33.1%, heritability 68.1%) and palea length (PVE_{total} 45%, heritability 58.5%) (Tables 1, 2). The major QTLs for achene length/achene shape and the two synergistic QTLs for palea length explain a high amount of the genetically determined phenotypic variance (achene length 72.6%, achene shape 80.3%, palea length 64.0%).

The F2 distribution for absence versus presence of spots (Fig. 2) is consistent with the segregation of one major gene with dominance for the spotted condition. QTL analysis by linear regression and interval analysis,

Table 3. Phenotypic variance explained (PVE), measured as the coefficient of determination (R^2), by QTLs found for achene shape (AL/AD) and achene length. R^2_{total} from the multiple regression analysis (MRA) is a measure for the total phenotypic variance explained (PVE_{total}). The effects of achene shape QTLs are composed of effects on achene length and achene diameter. Achene length QTLs are specific for achene length.

		Achene shape (AS)		Achene length (AL)		Achene diameter (AD)	
		R^2	p	R^2	p	R^2	p
Achene shape QTLs	AS02R	0.266	<0.0001	0.197	<0.0001	0.050	0.0264
	AS03R	0.134	0.0002	0.075	0.0063	0.035	0.0651
	AS06R	0.091	0.0024	0.021	0.1553	0.069	0.0086
	MRA	0.331	<0.0001	0.171	<0.0001	0.109	0.0009
Achene length QTLs	AL02R	0.169	<0.0001	0.275	<0.0001	0.001	0.7539
	AL03R	0.036	0.0617	0.130	<0.0001	0.011	0.3113
	AL04R	0.048	0.286	0.146	<0.0001	0.016	0.2164
	MRA	0.154	<0.0001	0.379	<0.0001	0.018	0.1880

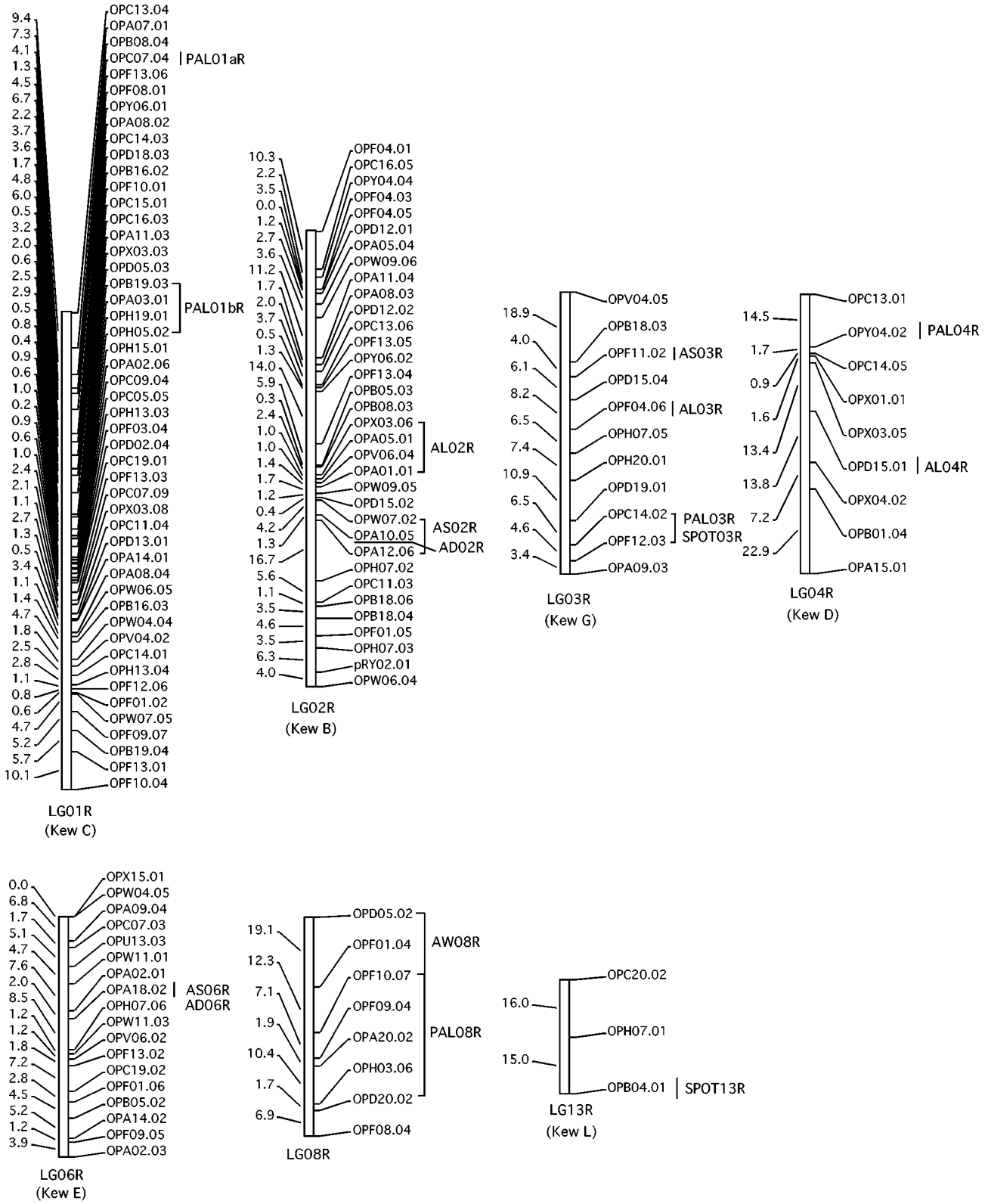

Fig. 3. Extract from the RAPD linkage map (Hombergen & Bachmann 1995). The strongest markers for achene characters are indicated by brackets or by lines (if only markers from one parent cosegregated with the character). Two different names for one QTL, e.g. in linkage groups 06R or 03R, indicate pleiotropic QTL effects, or close linkage. Names in brackets below the linkage groups represent corresponding linkage group names from an older RAPD map (Bachmann & Hombergen 1996).

however, did not reveal a strong QTL corresponding to this postulated locus. Only one marker from *M. douglasii* on linkage group 13R (OPB04.01, $R^2 = 0.153$, LOD 3.72; $\chi^2 = 12.3$, $p < 0.0001$, df. = 1) showed strong cosegregation with presence versus absence of spots.

For the strength of spotting (scored on a scale from 0 to 4), QTL analysis showed the same signal on linkage group 13R plus one additional QTL on linkage group 03R (SPOT03R = PAL03R) with partial dominance for *M. douglasii* (d/a = 0.357), and an effect on palea length that may be due to pleiotropy or linkage (Table 2, Fig. 3). Homozygosity for the *M. douglasii* allele in this modifying QTL is associated with achene pigmentation about twice as strong as in plants that are homozygous for the *M. bigelovii* allele (Table 2).

Discussion

Genetics of capitulum characters within the Asteraceae

Knowledge of the genetic basis of morphological differences between species is a prerequisite for their usage in phylogenetic studies (Bachmann 1998). Especially within the Asteraceae, characteristics of the capitulum and of the achene are important for the distinction and recognition of species (Sanders & Clark 1987, Bachmann & Battjes 1994, Jensen 1998). Several QTL mapping studies have been performed within the Asteraceae on species or population differences in capitulum characters such as the number of ray florets, involucral bracts, or the dimensions of the ligule (Bachmann & Hombergen 1996, Comes 1998, Moritz & Kadereit 2001, Kim & Rieseberg 1999). Kim & Rieseberg (1999) mapped QTLs for the length, width and shape of the achene in a cross between *Helianthus annuus* L. subsp. *texanus* Heiser and *Helianthus debilis* Nutt. subsp. *cucumerifolius* (Torr. & Gray) Heiser. They found four QTLs on two linkage groups for achene length, with the three strongest QTLs located on one linkage group (PVE from 15.2% to 18.1%). For achene width, four QTLs could be detected on four linkage groups with phenotypic effects from 5.9% to 17.1%, and for achene shape (achene length/achene width) six QTLs on five linkage groups (PVE 5.7% to 19.5%). The fact that mostly different QTLs are responsible for length, width and shape variation matches our observation of independent genetic systems for achene length and achene shape variation. For achene diameter our map resolution was possibly too low to map additional significant QTLs. These results point to the important aspect that the precise definition of the character is essential for the QTL effects found, and for their interpretation. A classical example is the study on cucurbit fruits by Sinnott (1935) who found polygenic inheritance for the length/width of the fruit, but Mendelian inheritance for fruit shape. For the shape of the leaf tip in an interspecific *Microseris* cross, monogenetic Mendelian inheritance could be detected (Bachmann et al. 1983). Leaf length, however, is governed by several genes with smaller effects (Bachmann & Hombergen 1996). Generally, if a shape character shows essentially two qualitatively different characters states (for example, leaf tip spatulate or pointed) the difference is typically determined by one or two genes only (for further examples see Gottlieb 1984).

Capitulum characters within *Microseris*

In the species of Section *Microseris* there is much morphological variation among populations, and there are hardly any clear diagnostic characters. Taxonomic treatment relies strongly on capitulum characteristics, and species are mainly recognized by the size and the shape of their ripe achenes (Chambers 1955, Bachmann & Battjes 1994). *M. douglasii* is different from the other annual *Microseris* species in showing relatively high variability in morphological characters within some populations (Bachmann & Battjes 1994, Bachmann & Roelofs 1995). In our study, variation in ten offspring plants (= 100 achenes) of our crossing parent *M. douglasii* was strikingly higher than in *M. bigelovii* offspring in the characters achene length, achene diameter and awn length, suggesting that responses to environmental variation (phenotypic plasticity) is higher in *M. douglasii* than in *M. bigelovii*. Such a variable expression of characters has been observed in natural populations of *M. douglasii* with five or fewer pappus parts even in different flowers within one flowering head (Bachmann et al. 1979), suggesting that a reaction norm for this character is inherited. Similar variable phenotypes could be produced in artificial hybrids between *M. douglasii* and *M. bigelovii* for the pappus part character (Vlot et al. 1992) and the number of microsporangia per anther (Gailing & Bachmann 2000). In controlled greenhouse experiments, however, heritabilities can be relatively high, sometimes near 100%, even for such a naturally variable character as the average number of achenes per flowering head (Bachmann & Battjes 1994).

Adaptive values for the achene characters studied here are not obvious. Since considerable genetic variation for practically all characters is hidden in natural populations by a high degree of phenotypic plasticity, it is likely that much of this genetic variation is effectively neutral. Even phenological characters seem to be determined mainly by plastic reactions. A significant shift in flowering time with latitude could be detected in *M. bigelovii* only over a North-South distance of about 1500 km (Bachmann 1992).

The apparently weak selection for fixed (ecotypic) differences within and among species is paralleled by our results on characters differentiating species. The plants are mainly autogamous, and certainly not dependent on pollinators. There seems to be no need for the reinforcement of species differences. Distribution ranges do not overlap completely, so that more or less pronounced species-specific autecological limitations together with the great rarity of cross pollination are probably sufficient to maintain species identity. Morphological differences among the species may be due mainly to drift. Our observation that characters used for species distinction (achene length and shape) show differences opposite to species differences in one achene length QTL and in one achene shape QTL underscores that the differences in these characters could have become much greater under appropriate selection. QTL studies have demonstrated QTLs with a polarity of the effects opposite to that in the parental strains in many cases, and revealed this as a source of agriculturally useful genetic variation (de Vincente & Tanksley 1993, Kim & Rieseberg 1999).

Of course such 'hidden' genetic potential can become a source of qualitatively or quantitatively new phenotypes in species hybrids in nature (Gottlieb & Ford 1988, Rieseberg et al. 1999, Schwarzbach et al. 2001).

QTL studies and evolution models

In recent years a growing number of QTL studies were performed on character differences in wild plants (Vlot et al. 1992, Hombergen & Bachmann 1995, Lin & Ritland 1997, Bradshaw et al. 1998, Hill & Doebley 1999, Gailing et al. 1999, Lauter & Doebley 2002). In a few model examples the evolution and genetics of diagnostic character differences have been examined (Gailing et al. 1999, Lauter & Doebley 2002). Such characters are often meristic, i.e. concerning the number of organs or organ parts. They are mostly invariant in natural populations of one species but discontinuously distributed between closely related species. In artificial hybrids, however, these characters become variable and accessible to molecular mapping studies. Multifactorial inheritance with one major gene and additional modifiers and strong epistatical interactions underlies the observed character variation in these cases (Vlot et al. 1992, Doebley et al. 1995, Gailing et al. 1999, Lauter & Doebley 2002). For example, in *Microseris* one major gene and three modifiers are responsible for the reduction from 4 microsporangia (MS) per anther to 2 MS. The modifiers have an effect only on the homozygous recessive (2 MS) genotype of the major gene, and can compensate for the effect of the major gene. Therefore, the mutation in the major gene alone has no visible phenotypic effect. In this way cryptic genetic variation in a discrete character might escape selection (Gailing et al. 1999, Lauter & Doebley 2002).

Generally, the genetics of characters that differ between species seems to follow no regular pattern, since the number and strength of interactions between different alleles (epistasis) vary widely (Orr 2001). For many quantitative and qualitative morphological character differences in wild species, however, one major gene and additional modifiers with smaller effects are responsible (Hombergen & Bachmann 1995, Lin & Ritland 1997, Bradshaw et al. 1998, Gailing et al. 1999, Gailing & Bachmann 2000, Moritz & Kadereit 2001). And even if the fraction of phenotypic variance explained by one QTL is relatively low, its substantial effect relative to the standing phenotypic variation within species is often high (True et al. 1997, Zeng et al. 2000). This is in contrast to the classical geometrical model by Fisher (1930) where the evolution of major phenotypic differences is the result of gradual divergence resulting from mutations of many genes with individual small and additive effects (Dobzhansky 1937, Coyne & Lande 1985, Orr & Coyne 1992). Based on the model proposed by Fisher (1930), Orr (1998, 1999, 2001) developed a simulation model for the evolution of adaptive characters that agrees with a genetic model of one major gene and additional modifiers. According to this model the phenotypic effects of genes fixed during response to selection are distributed exponentially.

The characters achene length and achene shape segregate in accordance with this model of one major gene and additional modifiers. For palea length five QTLs with intermediate and about equal additive phenotypic effects suggest a gradual divergence resulting from the mutation of several loci (Coyne & Lande 1985, Orr & Coyne 1992). However, the five QTLs already explain 45% of the total phenotypic variance and 77% of the genetically determined variance.

In contrast to discrete characters described above, epistatic interactions could not be detected for any of the achene characters examined. Orr (2001) points out that epistasis plays no (important) role in differences between species (Jones 1998, Zeng et al. 2000), although he quotes some exceptions for the quantitative character carotenoid concentration in *Mimulus* L. (Bradshaw et al. 1998) and the meristic character anal plate bristle number in flies of the genus *Drosophila* (True et al. 1997). We can speculate that for discrete character differences (for example, differences in the number of organs) epistasis plays a role in buffering the phenotype against genetic changes (Waddington 1942, Rendel 1959, Gailing et al. 1999, Lauter & Doebley 2002), whereas in quantitative character differences such as those measured here (metric characters such as length, width and perhaps shape) epistatic interactions between QTLs play a minor role for character evolution. The near lack of dominance for all QTLs concerning achene characters is in accordance with a model of

mutations with 'nondestructive' effects on physiological processes affecting the phenotype (Wright 1929, Orr 2001). Nevertheless, the strongest QTLs for achene length and for achene shape show major effects on the phenotype in terms of phenotypic variance explained, suggesting that a mutation with major effect on the phenotype does not necessarily have deleterious effects. On the other hand, mutations with small phenotypic effects might well have significant effects on fitness, for example for floral characters associated with the reproductive isolation of sympatric monkeyflower species (Bradshaw et al. 1998).

Achene pigmentation

In contrast to metric achene characters with continuous character variation in the F2 and F3, the bimodal distribution of achene pigmentation suggests the existence of one strong major gene. Derivatives of an intraspecific cross between *M. douglasii* (strain B13) with unspotted achenes and strain B16 with heavily spotted achenes showed 3:1 segregation with dominance for the spotted condition (Mauthe et al. 1985). Also, studies in flower pigmentation of *Clarkia gracilis* A. Nelson & Macbride and studies on the candystripe locus – which determines mutable pigmentation of the *Sorghum* L. leaf, flower and pericarp – indicated single-factor inheritance for presence versus absence of pigmentation, with presence of pigments dominant to absence (Gottlieb & Ford 1988, Zanta et al. 1994). Despite the 3:1 F2 frequency distribution suggesting the existence of one strong major gene, this major gene effect could not be found in our RAPD map. In order to find markers cosegregating with that major gene, additional RAPD and AFLP markers should be tested in a bulked segregant analysis (Michelmore et al. 1991) based on F2 DNA bulks.

Acknowledgements

We are grateful to Mrs B. Wedemeier for scoring the achenes. We also thank Mrs K. Lipfert and Mrs U. Tiemann for preparing Figure 1. We thank Prof. Dr. J. W. Kadereit and an anonymous reviewer for their helpful comments on the manuscript. O. Gailing was supported by the Deutsche Forschungsgemeinschaft (BA 536/ 11–1).

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