Acta Bot. Neerl. 42(3), September 1993, p. 255–268

Meristem geometry and heritable variation in numbers of florets and involucral bracts in *Microseris pygmaea* (Asteraceae, Lactuceae)

J. BATTJES*‡, N. O. E. VISCHER† and K. BACHMANN*

**Hugo de Vries Laboratory, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands and †Department of Molecular Cytology, University of Amsterdam, Plantage Muidergracht 14, 1018 TV Amsterdam, The Netherlands*

SUMMARY

Strains A92 and C96 of the Chilean annual, *Microseris pygmaea*, represent the genetically most divergent biotypes within the species. They differ, among other characters, in the numbers of florets and inner and outer phyllaries (involucral bracts) per head. Higher numbers in A92 are mainly the consequence of smaller primordia while the capitulum buds in both strains are about equal in size. Disruptive selection from a large F2 of the hybrid between A92 and C96 has produced lines with transgressive numbers of parts with non-parental correlations among numbers of parts. The early development of the capitula was studied in F4 families derived from two F2 plants selected for extreme organ number phenotypes. Differences in organ numbers among these families are primarily due to differences in capitulum size which did not differ significantly between the parental strains. Values for other parameters such as floret primordium size, height of the zone in which inner phyllaries arise, and difference in size between peripheral and central florets, did not significantly contribute to the observed differences in organ numbers. The multiple gene differences suggested by the transgressive segregation in the F2 can be partly traced back to various primary effects on meristem differentiation.

Key-words: Asteraceae, developmental constraints, inflorescence, meristic characters, ontogeny, quantitative traits.

INTRODUCTION

The annual species *Microseris pygmaea* D. Don (Asteraceae: Lactuceae) has originated in Chile after long-distance dispersal from western North America. The species occurs in isolated and morphologically distinct populations in central Chile (Bachmann *et al.* 1985). Populations of *M. pygmaea* differ, among other characters, in numbers of outer phyllaries, inner phyllaries and florets per capitulum. We will try to reconstruct the genetic events that underly the divergence in these quantitative characters. Plants from strain A92 and C96, which represent the most divergent numbers of parts within the species, have been crossed, and the segregation patterns in the F2 and F3 families of the hybrid have been

‡To whom correspondence should be addressed.

analysed (Bachmann 1991). Differences between the parental strains in numbers of outer phyllaries, inner phyllaries and florets per capitulum had a strong heritable component. The three traits are correlated, but the number of inner phyllaries per head is more or less constant over a considerable range of floret numbers. Moreover, where phenotypic variation occurs in the three traits, much of its genetic component is masked by plastic responses of the plants to their environment. Considerable differences in the amount of plasticity, the non-genetic variance, between homozygous strains interfere with a straightforward quantitative genetic analysis of the variation (Bachmann 1991).

Organ numbers in Asteracean capitula are not explicitly coded by genes but result from the interplay of genetically and environmentally determined sizes and shapes of meristems. For example, Palmer & Steer (1985) could predict the number of fruits on mature capitula in various sunflower strains from the size of developing capitula. The difference in the number of florets between strain A92 and strain C96 of *M. pygmaea* could be explained by differences in floret primordium size on roughly equally sized capitulum buds (Battjes *et al.* 1992). Here, we want to find out whether differences in floret primordium size are sufficient to explain the segregation of numbers of florets in the hybrid between strains A92 and C96. We also investigate whether the segregation of the numbers of inner and outer phyllaries is another expression of the size factor determining variation in floret numbers, or whether additional genetic factors can be demonstrated in the hybrids.

MATERIALS AND METHODS

Plant material

Five selected F4 families of hybrid E33 (A92 × C96b) and a family each of the two parental strains were raised in the 1990/1991 season. The F3 parents of the five hybrid families are E33 F3-84, -89, -96, -128 and -132. The first three are a progeny of E33 F2-120, selected for highest numbers of inner phyllaries, the latter two are from E33 F2-140, selected for lowest phyllary numbers among 205 F2 plants (Bachmann 1991). The five F3 parent plants were selected for differences in number of florets, and for disproportionally large differences in inner and outer phyllaries per head (Table 1). Plants from the seven families were derived by selfing and grown in two groups, one to harvest plants for meristem preparations, the other to harvest mature heads for phenotypic analysis (Table 1). They were germinated in Petri dishes and planted individually in pots on October 8, 1990 to obtain mature heads (group I), and October 15, 1990 for meristem preparation (group II). The pots were arranged randomly in a greenhouse. Temperature was not allowed to drop below 10°C, and no artificial light was applied.

Organ counts

Heads from the first group of plants were harvested at maturity and the numbers of outer phyllaries, inner phyllaries and achenes (= number of florets) per head were counted in 10 or 20 heads per plant.

Plants from the second group were harvested for meristem preparation. In addition, the numbers of outer phyllaries, inner phyllaries and florets per head of ten heads from each family were determined as a control for the meristem measurements.

Meristem preparation

Three plants were harvested for meristem preparation twice a week from each of the five hybrid F4 families and from the parental controls. Leaves were removed and the plants

Table 1. Parent–offspring values in selection experiments for numbers of outer phyllaries, inner phyllaries and florets per capitulum in *Microseris pygmaea*

Strain, family		Year	Number of plants (heads)	Florets*	Inner phyllaries*	Outer phyllaries*
A92 P1		1989‡	20 (144)	63·5±1·30	14·2±0·13	9·9±0·11
A92 P1		1991 (II)	4 (10)	65·3±1·16	15·3±0·44	10·8±0·14
A92 P1		1991 (I)	2 (20)	86·4±0·85	17·4±0·30	9·9±0·16
C96 P2		1989‡	18 (174)	37·9±0·59	11·5±0·06	7·7±0·02
C96 P2		1991 (II)	4 (10)	43·4±1·13	11·6±0·28	6·7±0·82
C96 P2		1991 (I)	3 (30)	61·1±0·75	12·8±0·00	7·2±0·49
E33 F2-120		1989	1 (10)	90·4	18·1	10·8
E33 F2-140		1989	1 (10)	33·8	9·3	7·5
E33 F3-84	(from F2–120)	1990	1 (10)	85·3	12·9	10·8
E33 F3-89	(from F2–120)	1990	1 (10)	89·9	18·9	11·3
E33 F3-96	(from F2–120)	1990	1 (10)	59·9	19·6	10·9
E33 F3-128	(from F2–140)	1990	1 (10)	32·0	10·0	7·7
E33 F3-132	(from F2–140)	1990	1 (10)	30·7	9·9	9·4
E33 F4-1	(from F3–84)	1991 (II)	3 (10)	63·6±1·80	13·9±0·26	9·9±0·22
E33 F4-1	(from F3–84)	1991 (I)	11 (110)	83·7±0·97	13·9±0·11	9·4±0·05
E33 F4-2	(from F3–89)	1991 (II)	3 (10)	85·5±4·44	18·4±1·16	10·7±0·42
E33 F4-2	(from F3–89)	1991 (I)	14 (110)	48·1±0·56	12·9±0·03	9·5±0·03
E33 F4-3	(from F3–96)	1991 (II)	4 (10)	64·5±2·40	13·8±0·20	10·0±0·22
E33 F4-3	(from F3–96)	1991 (I)	28 (280)	76·7±0·55	16·3±0·13	9·8±0·04
E33 F4-4	(from F3–128)	1991 (II)	4 (10)	43·0±2·24	12·3±0·37	7·0±0·24
E33 F4-4	(from F3–128)	1991 (I)	15 (250)	56·5±0·47	12·9±0·00	8·3±0·03
E33 F4-5	(from F3–132)	1991 (II)	4 (10)	45·6±1·33	12·8±0·00	7·6±0·10
E33 F4-5	(from F3–132)	1991 (I)	27 (370)	62·6±0·34	13·0±0·00	9·0±0·02

*Mean values of plant averages ±SE.
‡Data from Bachmann (1991).

were fixed in CRAF III for 1 week, passed through a graded ethanol series and stored in 70% ethanol. Plants were dissected under a dissecting microscope in 70% ethanol. From each family, ten capitulum buds at stage 5 (Battjes *et al.* 1992) were dissected. This is the stage at which the previously bare receptacle fills up with floret primordia in a centripetal progression. Inner and outer phyllaries were removed, and the capitulum was cut off just below the lowest flower primordia. Figure 1 shows an *M. pygmaea* head at stage 5. The outer phyllaries (OP) surround the head and are not associated with florets. The inner phyllaries (IP) surround the receptacle in a more or less continuous ring and always have florets in their axils ('peripheral florets', PF). Central florets (F) have no associated bracts. For measurements, capitula were stained with Schiff reagent without pretreatment in such a way that the primordia were clearly outlined, and embedded in Historesin glycol methacrylate. The detailed staining and embedding procedures are described in Battjes *et al.* (1993).

Measurements

Three-dimensional measurements on the whole mounts were made with the aid of a light microscope. Images were recorded with a video camera and displayed on a Macintosh IIci

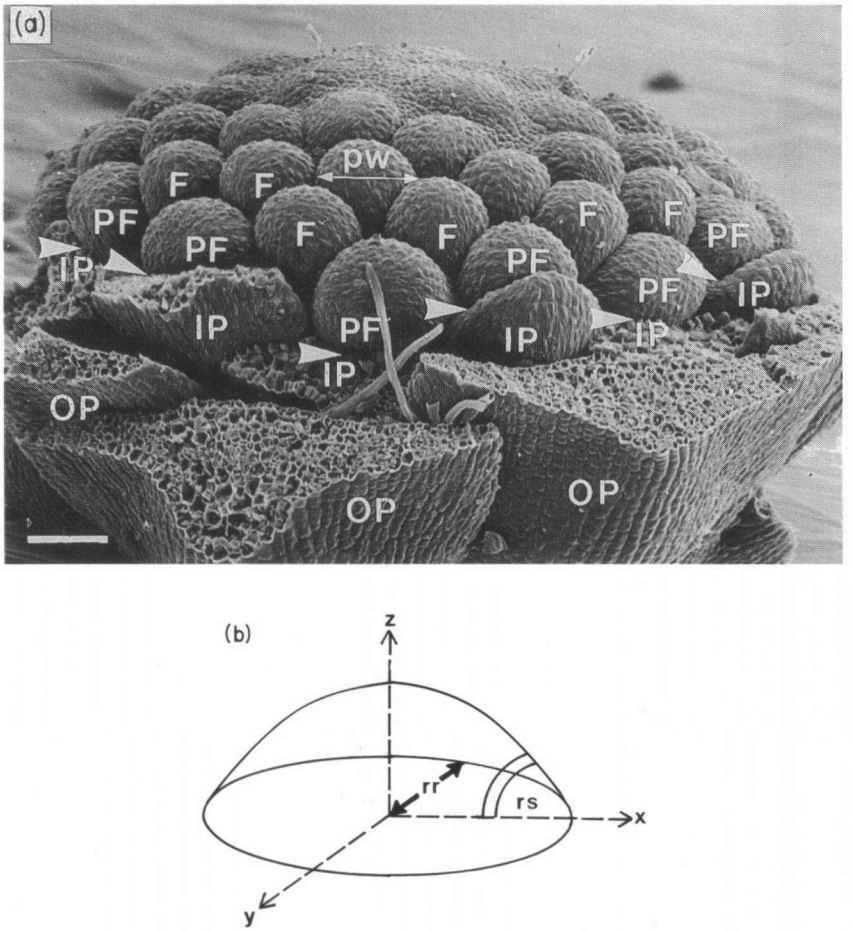

Fig. 1. Explanation of meristem measurements. (a) Scanning electron micrograph of an *M. pygmaea* head at stage 5 of development. OP: outer phyllaries; IP: inner phyllaries; PF: peripheral florets; F: florets; pw: primordium width. Arrows point to the border between peripheral florets and associated inner phyllaries. Note the difference in height between the borders ('lower border shift'). Scale bar: 500 μm. (b) Schematic drawing of receptacle at stage 5. rr: Receptacle radius; rs: receptacle slope.

computer as described in Battjes *et al.* (1993). The x, y, and z-coordinates were determined for four points around the circumference of each floret primordium to mark the broadest tangential and radial diameters. The presumed ontogenetic order of primordia was determined as described in Battjes *et al.* 1992. From the data matrix of each head we calculated the values of seven parameters which theoretically may influence the numbers of florets and inner phyllaries, as illustrated in Fig. 1.

Primordium width. Primordium width (pw) was calculated as the average width of all primordia on a head, and used as an estimate for primordium size.

Receptacle radius. The radius of the receptacle (rr) is partly correlated with the surface area of the receptacle, and thus partly determines the available space for floret primordia.

Slope of the receptacle. As a measure of shape, the receptacle slope (rs) was defined as the regression coefficient of primordium height on its distance from the centre of the head, for the first 40 primordia. In addition to this measure, we determined the relationship between height and radius of the receptacle in the heads which were almost filled up with florets (i.e. radius of the open part of the receptacle smaller than 100 μm).

Width ratio. Average width of the 13 peripheral florets (PF) divided by the average width of primordium 14 through 18 (F). A low width ratio implies a high number of primordia at the circumference and thus more inner phyllaries relative to the number of florets.

Lower border shift. The largest distance along the *z*-axis between the lower borders (arrows) of two primordia among the first 13. It estimates the width of the zone at the periphery of the head in which inner phyllaries are present. The larger the distance between two lower borders, the more inner phyllaries can be expected.

Surface/primordium ratio. Surface of the head (calculated from its radius) divided by the average surface of the floret primordia. The higher the ratio, the more florets will fit on the receptacle.

Circumference/primordium ratio. Circumference of the head (calculated from its radius) divided by the average width of the first 13 primordia. The higher the ratio, the more inner phyllaries will fit on the periphery of the head.

Statistics

The heritability of the numbers of florets and inner and outer phyllaries was determined from a regression of F4 offspring values on F3 parental values (Falconer 1989).

We applied ANOVA (analysis of variance) to the meristem data in order to test differences between the seven inbred families. When differences among all families were significant, we made the following planned contrasts (Sokal & Rohlf 1981). Parental strains were compared with the F4 progenies. Since the five F4 families were ultimately derived from two F2 plants, we contrasted the three F4 families derived from one F2 plant (F4-1, -2 and -3) with the two F4 families derived from the other F2 plant (F4-4 and -5). Additionally, we determined the differences among F4-1, -2 and -3, between F4-4 and -5, and between A92 and C96. ANOVA on floret numbers is based on data from group II plants. Variance of numbers of inner phyllaries was not analysed since their distribution is non-normal.

RESULTS

Genetics of mature capitula (plants of group I)

The aim of the large-scale selection experiment was to determine heritability of the numbers of inner phyllaries and florets in the five selected families in which we also determined the meristem parameters (see below).

The regression of floret numbers in F4 offspring on those of their F3 parent plants, i.e. their realized heritability, is not significant ($h^2 = 0{\cdot}09$). Genotype–environment interaction plays a considerable role in the determination of numbers of florets per head. This is evident from a comparison of data between plants from the same families germinated and planted 7 days apart in the same greenhouse. Especially F4-2 shows an almost two-fold

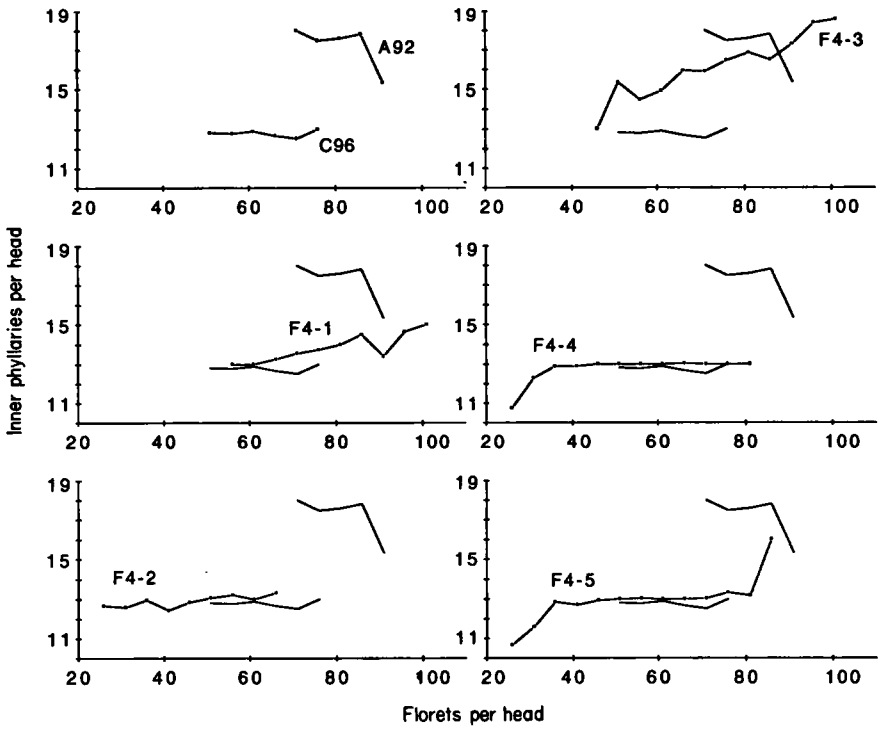

Fig. 2. Relationships between numbers of florets and numbers of inner phyllaries per head in two *M. pygmaea* strains (A92 and C96), and five F4 families derived from the hybrid E33 between A92 and C96. Points represent average values for classes of five florets. Only data points representing more than one head are shown. Values for parental strains A92 and C96 are repeated in all graphs for comparison with the F4 offspring families. Note the preference for 13 inner phyllaries in the 30–80 florets range in C96, F4-2, F4-4 and F4-5, and higher numbers of inner phyllaries in the same range of floret numbers in A92 and F4-3.

difference in floret numbers between plants of group I and II (Table 1). Heritability of the number of outer phyllaries per head is significant ($h^2 = 0{\cdot}37$; $P < 0{\cdot}05$). Heritability of inner phyllary number per head is low ($h^2 = 0{\cdot}18$, significance test not applicable).

The relationship between numbers of inner phyllaries, outer phyllaries, and florets shows differences among the F4 families. In four F4 families (F4-1, -2, -4 and -5) the number of inner phyllaries remains about 13 over a wide range of floret numbers (Fig. 2). Parental strain C96 has on average slightly fewer than 13 phyllaries in every capitulum size class, while A92 and F4-3 have many more (Fig. 2). Two inbred lines, F4-1 and -3, selected from one F2 parent, differ in the number of inner phyllaries in heads with the same number of florets. Thus, the number of inner phyllaries per head can vary independently from the number of florets per head. The selection of two lines from one F2 parent with roughly equal ranges of floret numbers but differences in outer phyllary numbers per head resulted in one line with low outer phyllary numbers (F4-4) and one with higher numbers (F4-5; Fig. 3).

Meristem parameters and control counts (plants of group II)

All meristem parameters, except slope of the receptacle, did show significant differences among the seven families (Table 2). The following comparisons were significant.

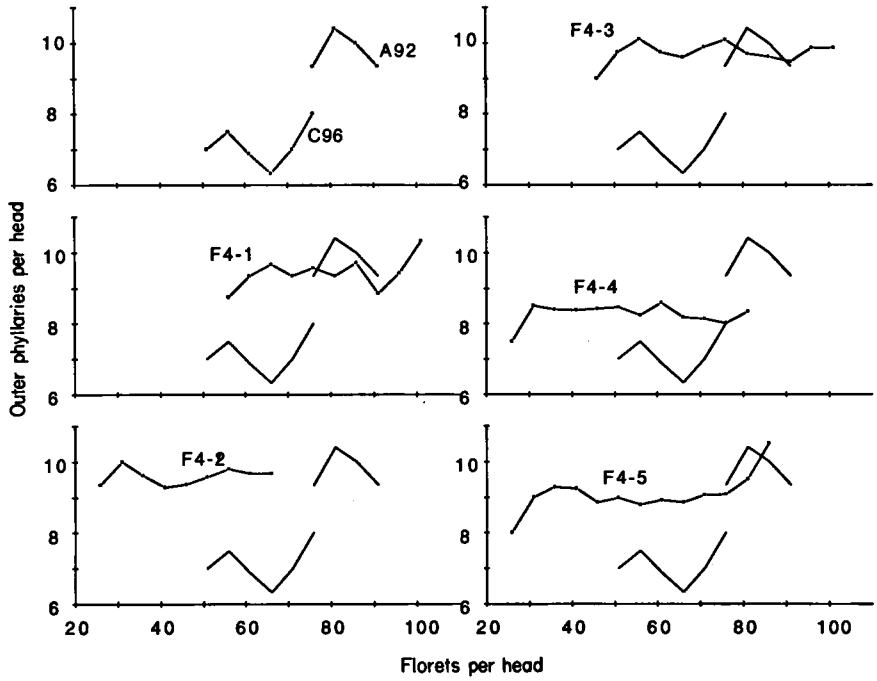

Fig. 3. Relationships between numbers of outer phyllaries per head and florets per head in A92, C96 and the five F4 families derived from hybrid E33. Data points represent average numbers of outer phyllaries in ranges of five florets. Values for parental strains A92 and C96 are repeated in all graphs for comparison with the F4-offspring families. Numbers of outer phyllaries do not increase over a wide range of floret numbers in all seven families. F4-4 and F4-5, selected for differences in numbers of outer phyllaries, differ in average numbers of outer phyllaries per head in classes of equal floret numbers.

Primordium width. Primordium width differed significantly between the parental strains (Table 2, Fig. 4a). The strain with the highest number of florets and inner phyllaries (A92) has the smallest primordia. F4-4 and -5 also differ from each other in primordium width although numbers of florets and inner phyllaries do not differ between these two families.

Receptacle radius. F4-1, -2 and -3 differ significantly from F4-4 and -5 (Fig. 4b, Table 2). The F4 families with the higher numbers of florets and inner phyllaries (F4-1, -2 and -3) also have larger receptacles. This is in contrast with the parental strains, in which the differences in organ numbers seem to be related to primordium size rather than receptacle size.

Slope of the receptacle. The shape of the peripheral part of the receptacle is depicted in Fig. 5a. All capitula are essentially conical at the periphery. The slope of the receptacle did not differ significantly among families. The average slope for all 70 heads is $-1{\cdot}00$ SE $0{\cdot}036$. Because the shape of the heads is similar among the seven families, we expect heads in families with small receptacles to be lower when their receptacles are filled up with primordia. This appears to be true from Fig. 5b: there is a significant linear regression of the height of the receptacle on the radius in heads which are almost filled with primordia

Table 2. Analysis of variance (sum of squares and significance of F-ratio) of meristem parameters and numbers of florets

Source	df	Primordium width	Receptacle radius	Width ratio	Lower border shift	Floret number	Surface/ primordium ratio	Circumference primordium ratio
		Sum of squares						
Among families	6	1066·4	21846·1	0·0817	2856·2	16030·8	1732·4	152·4
Planned contrasts:								
C96, A92 vs F4	1	22·0	254·0	0·0009	2·4	413·5	112·1	6·2
F4-1, -2, -3 vs -4, -5	1	121·6	18254·0	0·0251	1119·2	9329·8	1405·3	130·8
between C96, A92	1	617·3	1921·3	0·0080	692·7	2464·2	66·6	3·7
among F4-1, -2, -3	2	57·0	1385·7	0·0117	524·9	3781·3	133·2	10·3
between F4-4, -5	1	248·5	31·0	0·0359	517·2	42·1	15·2	1·5
Within families (error)	63	3422·0	9776·2	0·1751	4463·4	6215·3	5245·0	205·2
		Significance						
Among families		**	*	***	***	***	**	***
C96, A92 vs F4		ns	ns	ns	ns	*	ns	ns
F4-1, -2, -3 vs -4, -5		ns	***	**	***	***	***	***
between C96, A92		***	ns	ns	**	***	ns	ns
among F4-1, -2, -3		ns	ns	ns	*	***	ns	ns
between F4-4, -5		*	ns	***	*	ns	ns	ns

df: degrees of freedom.
ns: not significant; * $P<0{\cdot}05$; ** $P<0{\cdot}01$; *** $P<0{\cdot}001$.
Sums of squares of the planned contrasts add up to the among families sum of squares. A92, C96: parental strains. F4 families are from the hybrid strain E33 between A92 and C96. F4-1, -2 and -3 are from one F2 ancestor, F4 -4 and -5 from another, both selected for extreme organ number phenotypes.

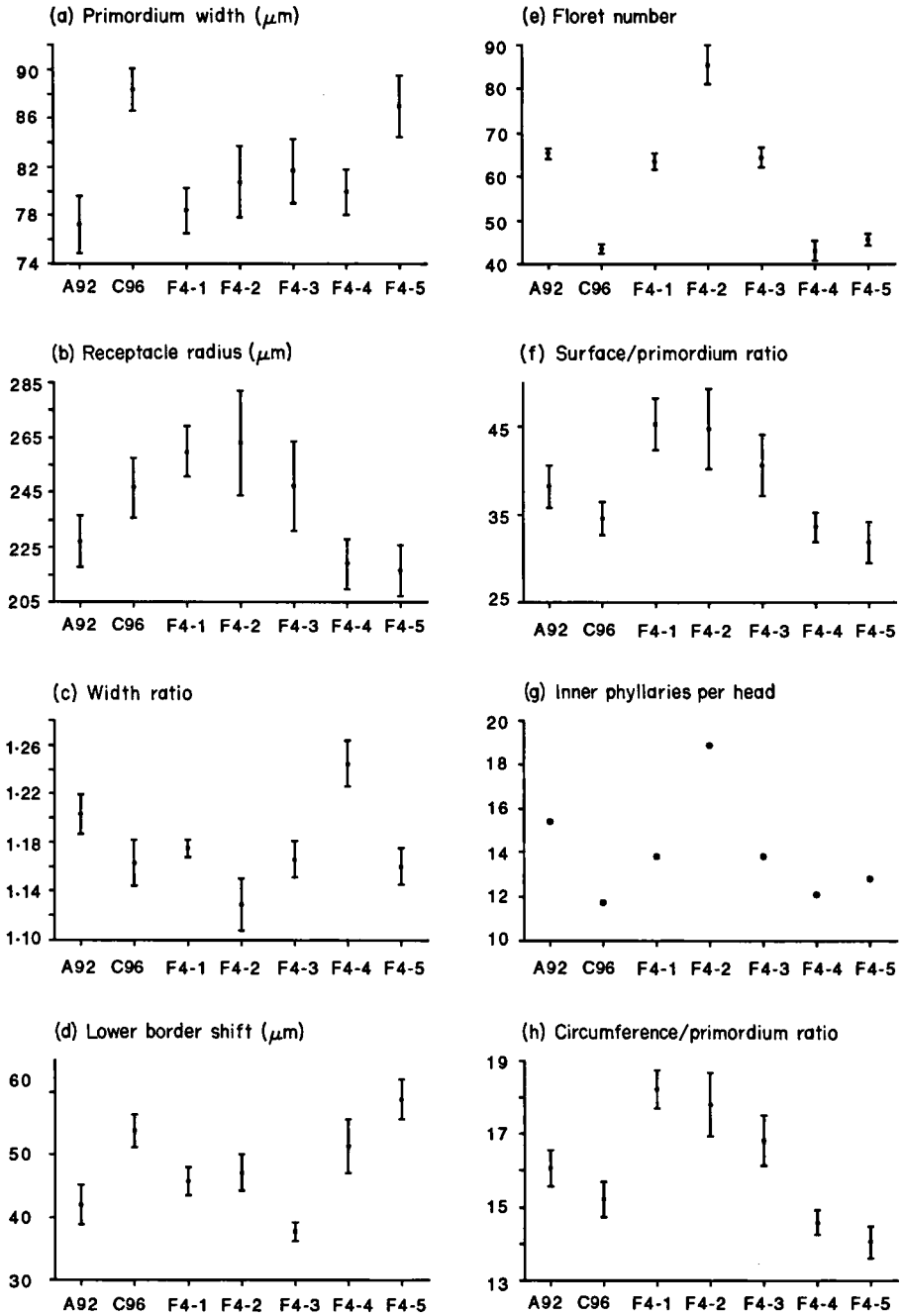

Fig. 4. Average meristem parameters and organ numbers in seven families. Bars represent standard errors. Differences in floret number between A92 and C96 (e) are related to variation in primordium width (a) whereas the difference between the progeny of F2-120 and F2-140 (F4-1, -2 and -3 vs F4-4 and -5), is mainly due to a difference in receptacle size. Variation in numbers of inner phyllaries per head (g) is not related to width ratio or lower border shift (c and d). Surface/primordium ratio (f) correlates well with number of florets. The correlation between circumference/primordium ratio (h) and numbers of inner phyllaries is less.

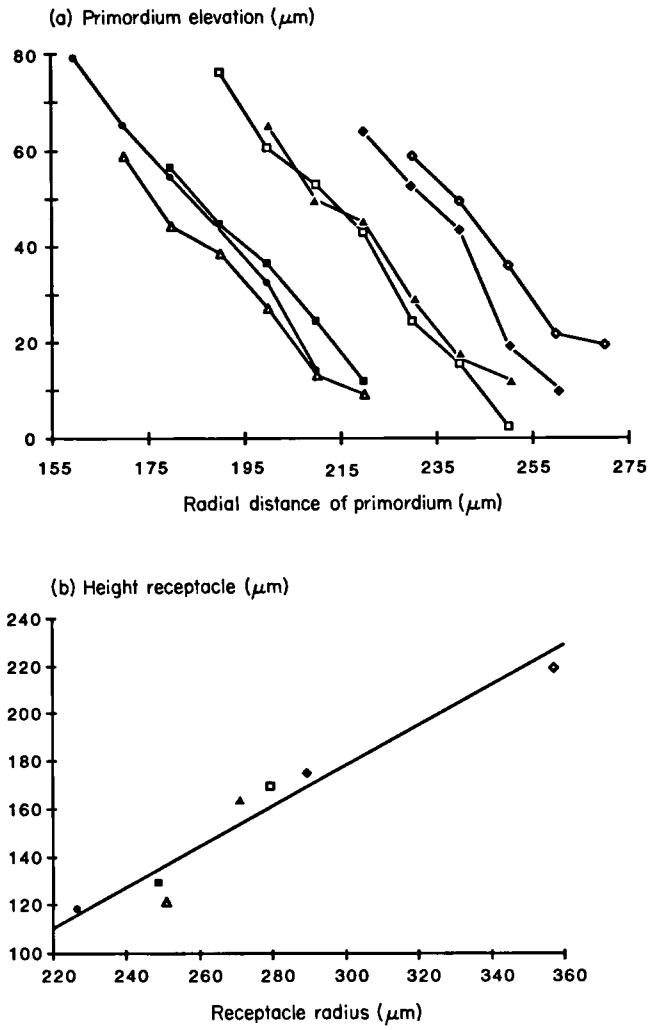

Fig. 5. Receptacle shape in A92 (■), C96 (□), F4-1 (◆), F4-2 (◇), F4-3 (▲), F4-4 (△), and F4-5 (●). (a) Relationship between radius and height of the first 40 primordia in ontogenetic order. Average values for 10-μm ranges of radius. The form of the head is similar among the seven families. (b) Relationship between receptacle width and receptacle height in heads almost filled with primordia.

($P<0{\cdot}001$). The regression coefficient (0·84) differs from the absolute slope of the head (1·00), probably because the head is flattened at the top.

Width ratio. The size of individual floret primordia decreases from the circumference of the head towards the centre, but this decrease is not continuous. Between the thirteenth and fourteenth primordia there is a sharp decline in primordium width in all families except F4-2 and -5 (Fig. 6). The change in width is estimated from the width ratio.

Families F4-1, -2 and -3 have a significantly lower width ratio than F4-4 and -5 (Table 2, Fig. 4c). Differences in width ratio are also found between F4-4 and -5. It is remarkable that the F4-1, -2 and -3 which were selected for their varying numbers of inner phyllaries in

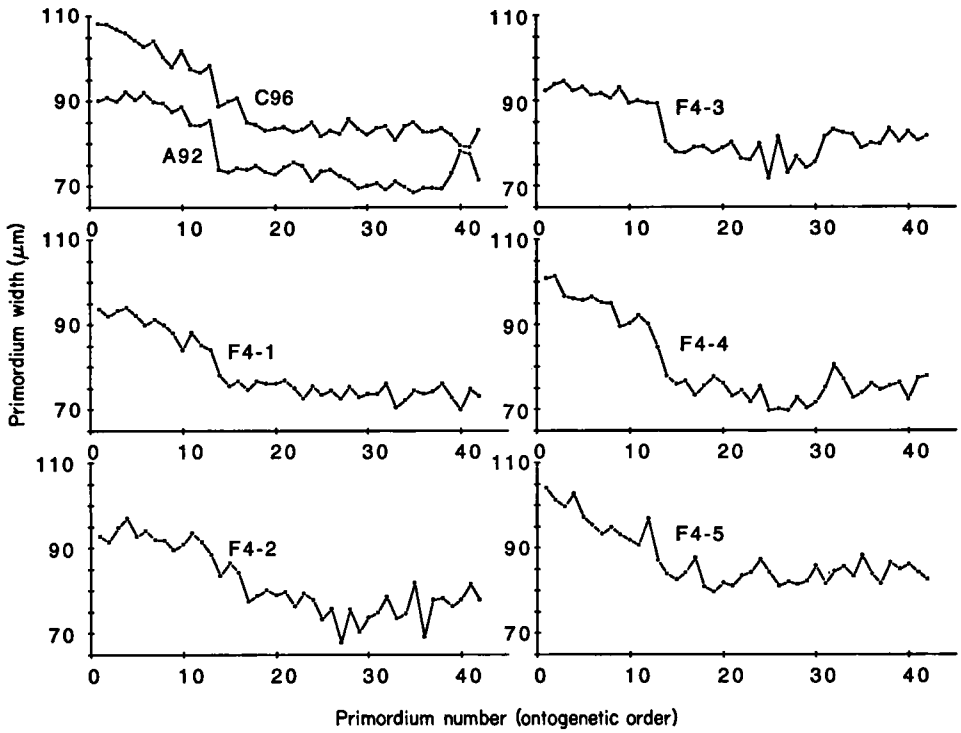

Fig. 6. Average primordium width per primordium number in seven families. In A92, C96, F4-1, F4-3 and F4-4, the thirteen most peripheral primordia are much wider than the primordia from number 14 onward, with a sharp decline between primordia 13 and 14. In F4-2 and -5, the transition in width between primordia 13 and 14 is smoother.

heads with equal numbers of florets, do not show differences in width ratio. The same holds true for the parental strains. In contrast, F4-4 and-5 have equal numbers of inner phyllaries but differ in width ratio. Therefore, variation in width ratio seems to have no relationship with differences in numbers of inner phyllaries.

Lower border shift. Significant differences exist between the parental strains (Fig. 4d, Table 2). Additionally, F4-1, -2 and -3 are significantly different from F4-4 and -5. Contrary to our expectation, families with relatively low numbers of inner phyllaries (C96, F4-4 and -5) have a larger lower border shift. This remains true when the lower border shift is divided by the receptacle radius, or when the lower border shift is measured for the first 8 instead of 13 primordia (data not shown).

Floret number. Differences in floret numbers in mature heads are significant for most comparisons made. Differences are not significant between F4-4 and -5 (Table 2, Fig. 4e).

Surface/primordium ratio. F4-1, -2 and -3 have a significantly higher ratio of receptacle to floret primordium surfaces than F4-4 and -5. This agrees with a higher number of florets in mature heads (Table 2, compare Fig. 4e with 4f). Correlation between surface/primordium ratio and number of florets per head is significant at the 5% level ($r = 0{\cdot}82$, $df = 5$).

Inner phyllary number. Since distribution of inner phyllaries is non-normal, no ANOVA was applied (Fig. 4g).

Circumference/primordium ratio. F4-1, -2 and -3 are significantly different from F4-4 and -5. As expected, families with the lower ratio (F4-4 and -5) have a lower number of inner phyllaries (compare Figs 4g and 4h). There is a positive correlation ($r = 0{\cdot}67$) between number of inner phyllaries per head per family and the ratio of capitulum circumference to primordium width.

DISCUSSION

The main objective of this paper is to identify differences in meristem size and shape which are involved in the expression of genetic differences in numbers of inner phyllaries and florets between A92 and C96. The difference in floret numbers between the parental strains A92 and C96 had previously been correlated with a difference in the size of the floret primordia (Battjes *et al.* 1992). Here, we compare the parental strains with offspring of plants with extreme high and low numbers of florets and inner phyllaries selected from a large F2 family of the interstrain hybrid, E33.

Genetics

The differences between the selected F2 plants were, on average, maintained in the F3 and F4 plants (Table 1). From the F3, we selected 5 families for recombinant associations between numbers of florets and numbers of phyllaries. The F3 parents of F4-1, -2 and -3 selected for large capitulum size had either similar numbers of florets but different numbers of inner phyllaries (F4-1 and -2), or similar numbers of inner phyllaries but unequal numbers of florets (F4-2 and -3). Parents of F4-4 and -5, selected for small capitula, had equivalent floret and inner phyllary numbers but varied in the number of outer phyllaries. Recombinant numbers of florets and inner phyllaries probably have a genetic component, because the F4 families differ in these traits. In addition, these numerical traits are highly sensitive to small changes in environmental parameters. This is indicated by divergence in numbers between plants grown at different times in the same greenhouse (groups I and II) and explains the low heritability between seasons in the F3/F4 comparison. In our examination of meristem parameters in the F4, therefore, we focus on phenotypically less plastic variation and compare F4-1, -2 and -3 with F4-4 and -5.

Relation between meristem geometry and numbers of florets and phyllaries on mature heads

Florets. Light microscopic measurements confirm the result of previous measurements on SEM photographs that the difference in floret numbers between strains C96 and A92 is essentially due to smaller primordia on receptacles of equal size. When plants with extreme floret and phyllary numbers are selected from the F2 of a hybrid between the two strains, a different pattern is seen. The segregation of floret numbers between the two groups of F4 families is mainly due to equally-sized primordia on receptacles with different radii. Thus, the similarity of receptacle sizes between A92 and C96 hides a difference in the genetic determination of receptacle size that is exposed by disruptive selection from the hybrid F2. The combined effect of primordium width and receptacle radius on floret number was estimated ('surface/primordium ratio') and it was significantly correlated with actual floret numbers. The shape of the head, i.e. slope of the receptacle, was not

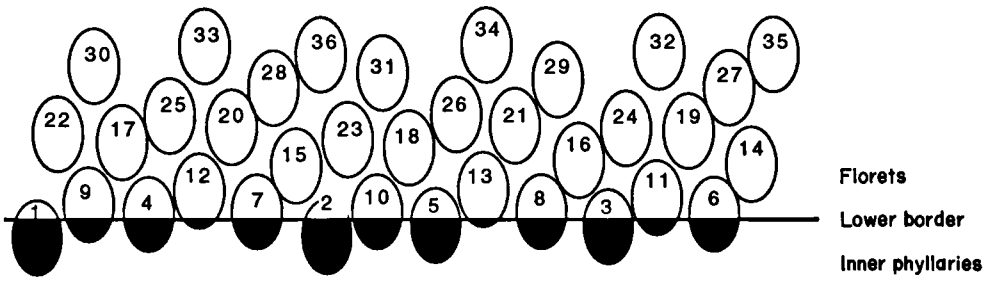

Fig. 7. Relative position of the first 36 floret primordia, average of 70 heads: elevation (*z*-axis) and angular position (as unrolled cylinder). A line (circle around the receptacle: lower border) indicates how the peripheral 13 primordia can be divided into floret and phyllary parts.

examined previously. It is essentially the same in all parental and F4 families. Variation in floret number apparently is not influenced by the slope of the head.

Inner phyllaries. Numbers of inner phyllaries in *M. pygmaea* are related to floret numbers but the relationship is not linear (Fig. 2). In heads with more than 80 or fewer than 30 florets per head, the number of inner phyllaries is strongly dependent on the number of florets. In parent strain C96 and most hybrid families, capitula with about 30–80 florets have preferentially 13 inner phyllaries. This is an example of numerical canalization (Fig. 2; Bachmann 1991). Anatomically, inner phyllaries are at the circumference of a receptacle which is otherwise filled with florets (Fig. 1). Each inner phyllary arises together with a peripheral floret from a common primordium (Battjes *et al.* 1992). The circumference/primordium ratio is an estimate of the number of primordia that will fit on the circumference of the head. Correlation between the circumference/primordium ratio and the number of inner phyllaries on mature heads was low, probably because this estimate considers only close packing without regard to spiral phyllotaxis. Numerical canalization of inner phyllary numbers in the 30–80 floret range in *M. pygmaea* results from an interaction of spiral phyllotaxis and close packing of primordia (Hirmer 1931; Fowler *et al.* 1992; Battjes *et al.* 1993). As a consequence of this interaction, often a Fibonacci-number (1, 2, 3, 5, 8, 13, . . .) of primordia will fit on the receptacle rim, in spite of variation in either receptacle or primordium size. This assumption is realistic in that primordium 14 generally lies much higher and closer to the centre of the head than primordium 13 (Fig 7; Battjes *et al.* 1993). If there is a border which divides primordia into a phyllary and a floret half, this border will have much more chance to cut through both primordium 1 and 13 than through both primordia 1 and 14 (Fig. 7). The fact that values for the lower border shift differ from zero (Fig. 4d) indicates that the border does not have a fixed position on the head as in Fig. 7 but shows local variation which probably is related to the ontogenetic order of the primordia.

The difference between A92 and C96 in the number of inner phyllaries on heads with equal numbers of florets could result from variation in this lower border shift. This parameter describes the extension of the zone in which inner phyllaries arise. However, it is not correlated with actual inner phyllary numbers. Although A92 has more inner phyllaries than C96, it has the lower value for this parameter. Another parameter which could affect the number of inner phyllaries is the width of the peripheral primordia. Because A92 has more inner phyllaries than C96, we would predict smaller primordia at

the periphery in A92. However, the relative width of the peripheral primordia (= width ratio) in A92 is not significantly different from that in C96. Additionally, it does not differ among F4-1, -2 and -3, which show considerable variation in the number of inner phyllaries.

Relative differences in numbers of inner phyllaries per head may be related to the fate rather than the initial number of subdivided peripheral primordia. We have shown previously that phyllary primordia arising from common primordia are progressively smaller at more central positions. From primordium 14 onward they can be very small and may not grow out into mature organs (Battjes *et al.* 1992). Possibly the probability that a very small primordium will grow into a phyllary differs among families. Inner phyllaries without associated florets at the phyllotactic position of a floret are found more often in C96 than in A92 (Battjes *et al.* 1992). This suggests that the interactions between the developing floret and inner phyllary at a phyllotactic site may differ between families.

Outer phyllaries. The number of outer phyllaries is relatively independent of the total number of florets on the head across the range of capitulum sizes in the families studied. At the same time, this number is variable at all capitulum sizes. As a consequence, the overall distribution of numbers of outer phyllaries per head does not deviate significantly from a normal distribution (data not shown), nor are certain numbers of outer phyllaries over-represented in all or most strains. In contrast, the distribution of inner phyllary numbers is strongly skewed and shows peaks at Fibonacci numbers (this study; Bachmann *et al.* 1985). Therefore numerical canalization is less obvious for the number of outer phyllaries than for inner phyllaries. Outer phyllary primordia are formed below the receptacle on the inflorescence axis at stage 1 and 2 of development, and give rise only to a phyllary, unlike the inner phyllary primordia (Battjes *et al.* 1992). The more variable geometry of the underside of the capitulum and the single developmental fate of primordia arising there may explain the weak correlation of the number of outer phyllaries with other meristic parameters of the head. We have no data on primordium sizes which may clarify the anatomical interdependence of numbers of outer phyllaries and florets.

ACKNOWLEDGEMENTS

We thank G.E.M. Hendriok and H.B.L. Lie for the counting of most of the heads.

REFERENCES

Bachmann, K. (1991): Genetic variation for meristic characters of the capitula of *Microseris pygmaea* (Asteraceae: Lactuceae). *Biol. Z bl.* **110:** 145–156.

Bachmann, K., Chambers, K.L., Grau, J. & Price, H.J. (1985): Genetic variation in *Microseris pygmaea* (Asteraceae–Lactuceae). *Beitr. Biol. Pfl.* **60:** 51–88.

Battjes, J., Bachmann, K. & Bouman, F. (1992): Early development of capitula in *Microseris pygmaea* D. Don strains C96 and A92 (Asteraceae: Lactuceae). *Bot. Jb. Syst.* **113:** 461–475.

Battjes, J., Vischer, N.O.E. & Bachmann, K. (1993): Capitulum phyllotaxis and organ number canalization in *Microseris pygmaea* (Asteraceae: Lactuceae). *Am. J. Bot.* **80** (in press).

Falconer, D.S. (1989): *Introduction to Quantitative Genetics.* Wiley, New York.

Fowler, D.R., Prusinkiewicz, P. & Battjes, J. (1992): A collision based model of spiral phyllotaxis (SIGGRAPH '92 Proceedings). *Computer Graphics* **26:** 361–368.

Hirmer, M. (1931): Zur Kenntnis der Schraubenstellungen im Pflanzenreich. *Planta* **14:** 132–206.

Palmer, J.H. & Steer, B.T. (1985): The generative area as the site of floret initiation in the sunflower capitulum and its integration to predict floret number. *Fld Crops Res.* **11:** 1–12.

Sokal, R.R. & Rohlf, F.J. (1981): *Biometry.* Freeman, San Francisco.