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Genetics of the proportion of peripheral yellow achenes on the capitula of *Microseris douglasii* strain D37 (Asteraceae, Lactuceae)

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SUMMARY

Microseris douglasii strain D37 is an interpopulation hybrid. The mature capitula in both parental strains bear three morphologically different kinds of fruits: peripheral hairy achenes (11·5% of all achenes in both strains) followed by a ring of non-hairy yellow achenes and a group of central achenes. The proportions of yellow achenes differ between the strains as a consequence of an allelic difference at a single locus. Lines homozygous for the two alleles of this locus in various recombinant backgrounds have been selected in the hybrid strain, and the influence of environmental and genetic variation, especially for the total number of achenes per capitulum, on the expression of the main gene is demonstrated.

Key-words: Asteraceae, developmental genetics, differentiation gradient, heterocarpy, inflorescence, *Lactuceae, Microseris*, recombinant inbreds.

INTRODUCTION

The differentiation of organs in higher plants often depends on the position of the organ primordium with respect to the plant apex. The apical meristem sequentially produces a number of primordia which develop into the basic rosette leaves, stem leaves, flower bracts and flower parts. Comparable organs on side branches are formed under the control of their own apex but are still more or less influenced by the position of the branch on the main axis. Similarly, within an inflorescence flowers may differ morphologically depending on their position (Froebe 1964; Weberling 1981). This is frequently the case for the compact inflorescence of the Asteraceae, in which morphologically different flowers and fruits tend to be arranged in concentric rings. The contrasting differentiation of ray florets around the rim and disc florets in the centre of capitula of daisies and sunflowers is the most striking example. In the genus *Microseris* of the Lactuceae only ray florets are formed. However, the peripheral florets still usually bear a violet stripe on the ligule, and the peripheral achenes usually have a hairy fruit wall and a lighter ground colour, so that at least two types of florets and two types of achenes can be distinguished.

Heritable variation in the concentric differentiation pattern of composite capitula is frequent in nature. This may be variation of the kinds of characters that differ along the radius of the capitulum (qualitative) and it may also be variation in the proportion of florets and achenes that have peripheral versus central differentiation (quantitative). In *Microseris douglasii* for instance, strains have been collected from nature in which the

relative numbers of hairy peripheral achenes vary all the way from plants with only hairy achenes to plants with no hairy achenes at all. This variation is not continuous. Due to the spiral phyllotactic arrangement of the flower primordia on composite heads (Pomplitz 1956; Bachmann & Chambers 1981; Hernandez & Palmer 1988) the proportion of peripheral fruits are powers of the golden ratio, $f=0{\cdot}618$, which is the limit of the ratio between two subsequent Fibonacci numbers. Fibonacci numbers form the series of 0, 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, . . ., in which each number is the sum of the two preceding numbers. Fibonacci numbers play an important role in the geometry in which plant organs are arranged (Rutishauser 1981; Jean 1984). In order to obtain a linear series of phenotypic values, we usually take the logarithm base 0·618 of the proportion of peripheral achenes which we call the lf value. An lf-value of 4·0 means that 14·6% of all achenes have a peripheral morphology and the absolute number of peripheral achenes is four Fibonacci numbers apart from the total number of achenes (e.g. 8/55).

Strains with various proportions of peripheral achenes can be crossed and the genetic differences between the differentiation patterns of these strains can be analysed. Bachmann (1983) has presented a model to explain the various patterns across the capitulum in which genes that determine the different floret and achene characters respond independently in a cell-specific way to the local concentration of some morphogen that is higher at the rim of the developing capitulum and decreases towards the centre. This model is purely hypothetical but it accounts for much of the variation seen when three or more different characters are concentrically distributed on a single head. If in two strains the proportions of several such comparable achene types all differ by the same ratios this could be interpreted as genetic differences in the gradient strength (Bachmann *et al.* 1985). If some characters change in proportion while others remain constant this could be explained as genetic variation in the response threshold of the genes that determine the character. Mauthe *et al.* (1984) have reported the first genetic segregation of this type. They worked with two inbred lines of *M. douglasii*. In both strains the peripheral achenes are hairy with a yellow ground colour. The yellow colour extends further towards the centre than the hairiness, so that there is a ring of non-hairy yellow achenes inside the ring of hairy achenes; the centrally located achenes are white with a layer of wax in one strain (D40) and brown with black spots in the other strain (B14). The proportion of hairy achenes is the same in both strains (11·5%; lf=4·5) and there is no segregation for this character in the F2 of their hybrid (D37). The proportions of yellow achenes, however, vary between the two parental strains and segregate in the F2, suggesting that only one major gene with two apparently additive alleles determines the proportion of yellow achenes in this strain. We have now raised F3 and F4 offspring of selected F2 plants of hybrid D37 with the aim of characterizing the phenotypic expression of this gene, especially against a background of plastic and genetic variation in the overall numbers of achenes per head, and of obtaining recombinant inbred plants homozygous for the main gene but varying in genetic background.

MATERIALS AND METHODS

Strain D37 is derived from an intraspecific cross between two inbred lines of *M. douglasii*. One strain, B14, originated from a natural population in Parkfield, Monterey County, California, the other strain, D40, came from Cholame, San Luis Obispo County, California. The parent plants were hand-pollinated by H.J. Price in 1981. Seed formed

after selfing of a hybrid plant was obtained from H.J. Price and an F2 of 30 plants was grown in 1983 (Mauthe *et al.* 1984).

In 1986, seeds obtained by selfing of eight selected F2 plants were set to germinate and F3 families of 30–40 plants were raised, scored for several morphological characters, and the ripe fruiting heads were harvested in April 1987.

Plants from four of these F3 lines were selected as parents of 10 F4 lines grown in the 1987/1988 season.

At the same time another 10 F2 plants were selected to produce F3 lines of, on average, 25 plants. Offspring of one F2 plant (F2–30) was raised in both years. Further selection into the F4 was performed in the offspring of F2 plant number 1 (F4 lines B and C), number 4 (F4 lines D, E, F and G), number 14 (F4 lines H and I) and number 16 (F4 lines K and L).

Seeds were germinated in autumn at room temperature on moist filter paper after a few days at 4°C and the seedlings were planted individually in 10-cm diameter clay pots. The plants were raised in a cool greenhouse (temperature not below 10°C) under natural day–night cycles. Although the conditions in the greenhouse were kept as uniform as possible, the environment varies from year to year, especially the amount of sunshine during the winter months. Therefore, each year a number of plants from both parental strains were raised together with the hybrids as controls to monitor the effect of environmental influences on the characters studied. The results reported here are based on a total of 874 hybrid specimens and 58 parental controls, obtained after selfing. After harvesting the mature capitula in spring, the numbers of hairy yellow, non-hairy yellow and central achenes were determined from the first three capitula of each plant.

RESULTS

The F3 and F4 lines add several new aspects to the analysis of the genetic determination of the proportions of yellow achenes. The fertility of the plants strongly decreased in the seasons when the F3 and F4 generations were grown; many plants produced heads with a high percentage of aborted fruits. This was most likely an environmental effect. The F2 plants were grown in a greenhouse in Heidelberg, F3 and F4 plants in Amsterdam. The sterile hairy achenes could still be recognized but the non-hairy aborted achenes could not be discriminated on colour. Therefore, capitula with less than 60% fertile achenes were excluded from the evaluation and the proportion of yellow achenes based on fertile achenes only. This differs from the evaluation of the F2, and we have recalculated the F2 data on the basis of the fertile achenes only (Fig. 1).

Figure 1a shows the data of Mauthe *et al.* (1984) on the segregation of the lf-values of the proportion of yellow fruits of the F2 generation and their parental controls. There appeared to be three phenotypic groups. Seven plants (23·3%) were overlapping the range of values found for the B14 parent (plant number 31 to 30 in Fig. 1a). Numbers in Fig. 1 indicate the plants that were selected for further breeding. Figure 1b shows the same data recalculated with the sterile achenes excluded. The absolute range of lf-values was not affected by this procedure but the plant numbers indicated that with the exclusion of sterile achenes from the total most plants shifted more or less towards lower lf-values, i.e. a higher proportion of yellow achenes. The overlap of 1/4 of the F2 phenotypes with that of the B14 parent was still evident, but the intermediate and low value phenotypes form a single group.

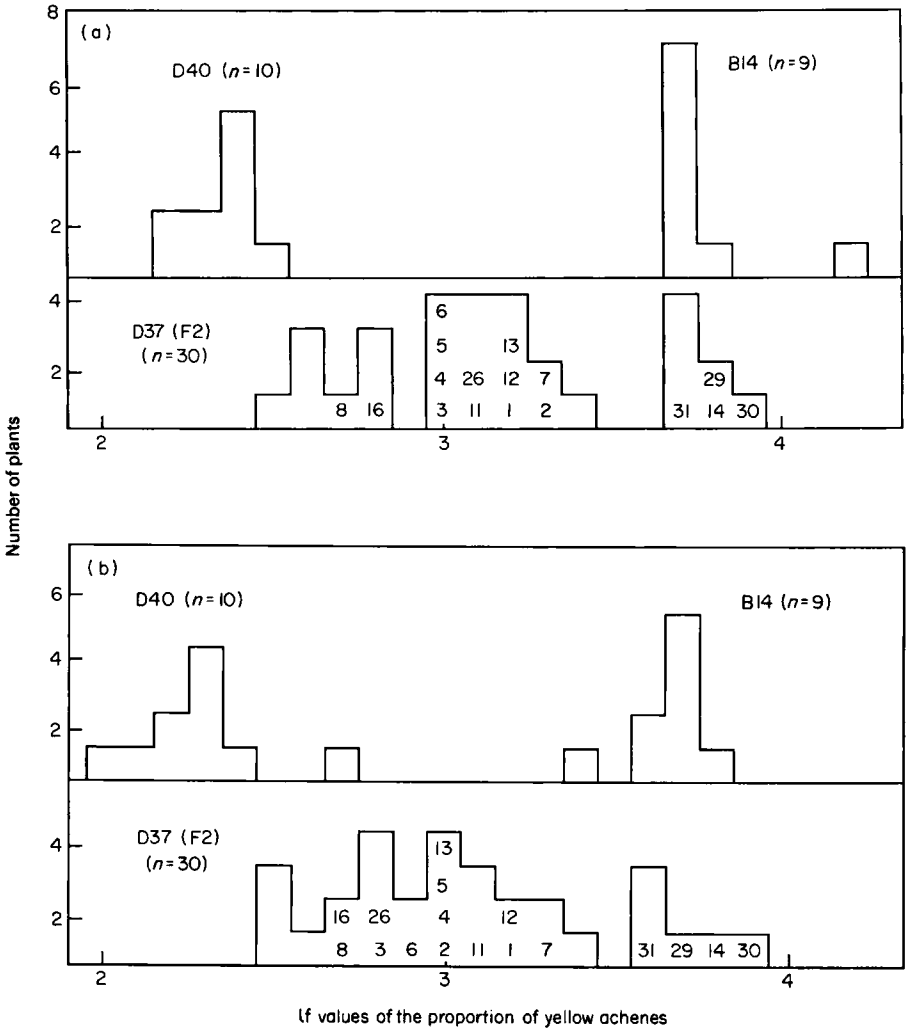


Fig. 1. Proportion of yellow achenes (logarithmic) in the parental strains, D40 and B14, and the F2 of their hybrid, D37. F2 plants used as parents of F3 lines are numbered. (a) Proportions including sterile achenes (Mauthe *et al.* 1984), and (b) proportions excluding sterile achenes.

We found that various factors influence the phenotypically expressed proportion of yellow achenes so that the single-gene genotype of a plant for this character can only be determined with certainty from the segregation in the offspring. Figure 2 shows the segregation patterns of the eight F3 lines raised in 1986/1987 and Table 1 shows the mean lf values and their variances for these lines and those for the 10 F3 lines raised in 1987/1988. Offspring of F2 plants 14, 29, 30 and 31 represent the homozygous B14 genotype for the proportion of yellow achenes. High mean values were indeed recovered in the F3 lines in both years. There was, however, a rather wide distribution of mean lf-values in these lines and the phenotypic distributions in all F3 lines were broad and continuous. Of the 17 F3 lines only those from F2 plants 6 and 8 showed phenotypic distributions that suggested that the F2 parent was homozygous for the D40 allele of the gene postulated by Mauthe

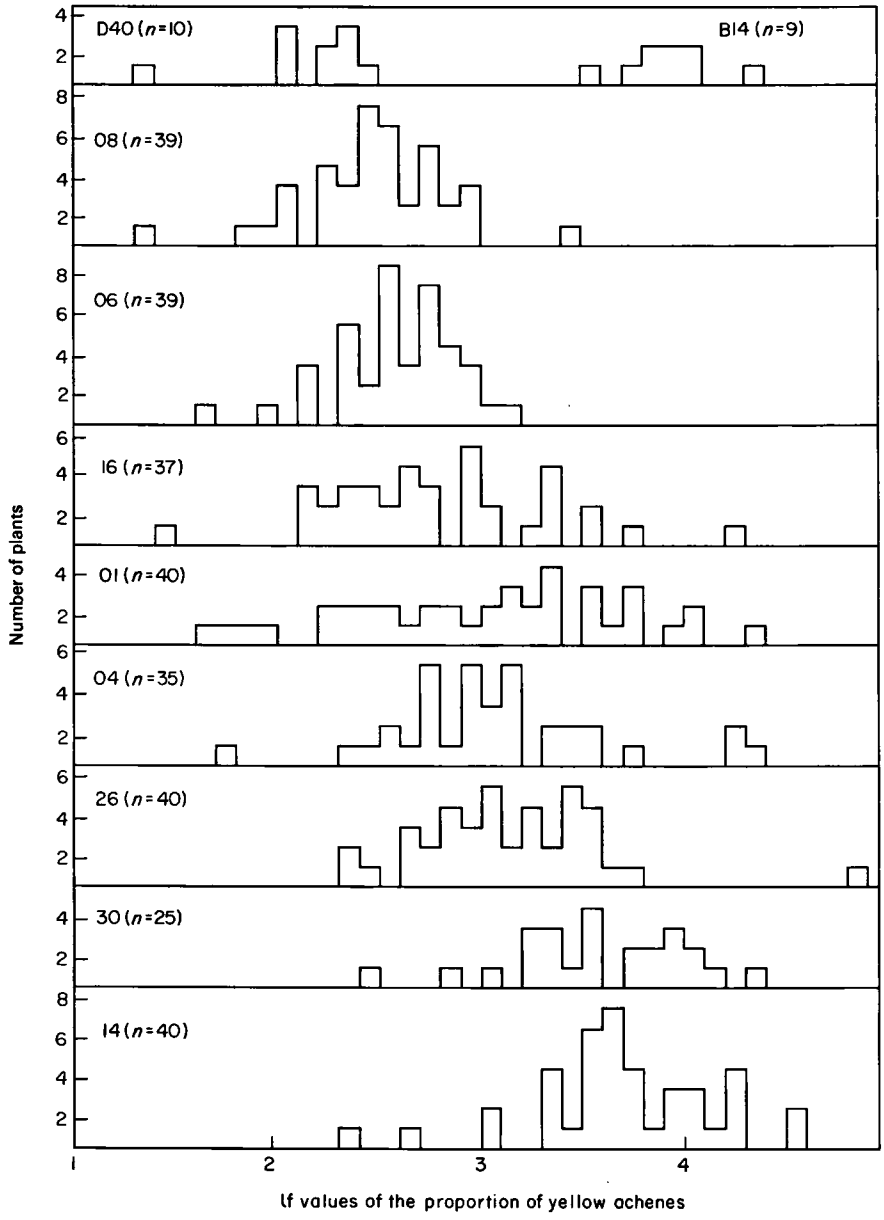


Fig. 2. Segregation of the proportion of yellow achenes (logarithmic) in eight F3 lines and the parental controls, raised in 1986/1987. Line numbers refer to the F2 parent plants (see Fig. 1).

et al. (1984). F2 plants 3, 26 and 16 also had lf-values in the low range but their F3 offspring clearly segregated across the whole range of lf-values. The intermediate and low lf values from heterozygous plants therefore overlap those of plants homozygous for the D40-allele and the F2 segregation is essentially a 3:1 rather than an additive 1:2:1 segregation. Moreover, the effect of the main gene was influenced by the environment and possibly by other genetic factors. We investigated other factors in order to determine to

Table 1. Mean lf-values and variances of F3 lines of 1986/1987 and 1987/1988

F3 1987	D40	08	06	16	01	04	26	30	14			B14
Mean lf	2·11	2·43	2·53	2·74	2·96	3·03	3·10	3·58	3·64			3·86
σ^2	0·09	0·14	0·09	0·29	0·45	0·29	0·20	0·27	0·20			0·05
F3 1988	D40	05	02	12	03	11	07	13	31	29	30	B14
Mean lf	1·95	2·49	2·78	2·90	2·97	2·99	3·08	3·16	3·24	3·51	3·68	3·78
σ^2	0·07	0·27	0·15	0·44	0·34	0·20	0·30	0·15	0·10	0·18	0·06	0·12

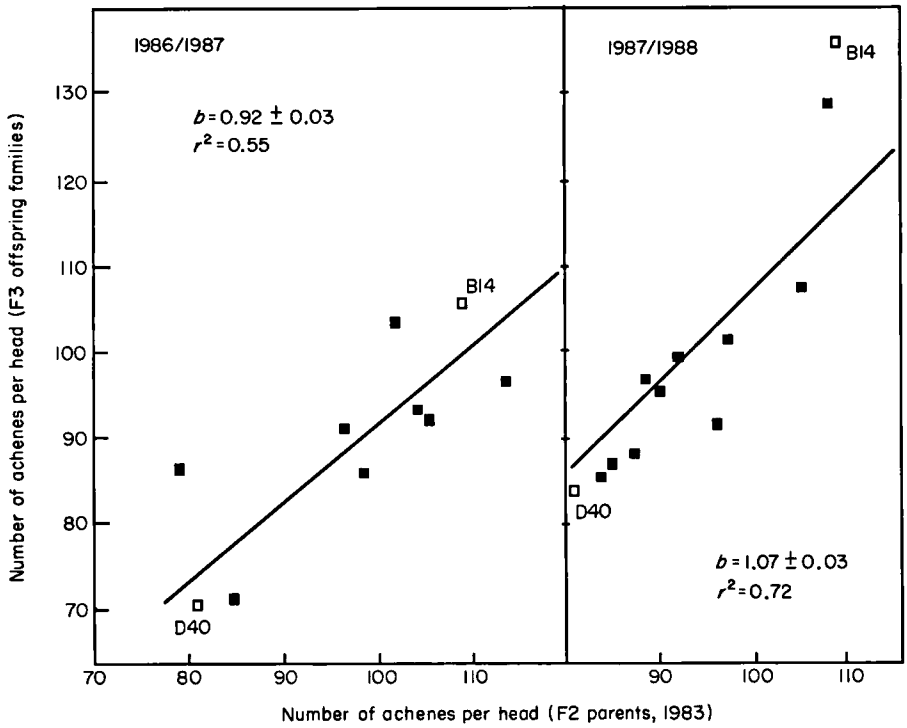


Fig. 3. Heritability of the number of achenes per capitulum: regression of F3 line means against F2 parent plant values in two subsequent seasons. Parental control values are represented as (□). b = Regression slope, r^2 = adjusted coefficient of determination.

what degree further genetical differences between the parents that segregate in the offspring lines influence the proportion of yellow achenes. The two parental strains differ consistently in the total number of achenes per head, and we found that both genetic and plastic variation in the total number of achenes had an influence on the proportion of yellow achenes. In order to find out to what extent the difference in the total number of achenes per head between the parental strains is genetically determined, we compared the average achene numbers in the F3 lines with those of the F2 parent plants (Fig. 3). The regression slopes (b), which represent the realized heritability are near 1·0 and the adjusted

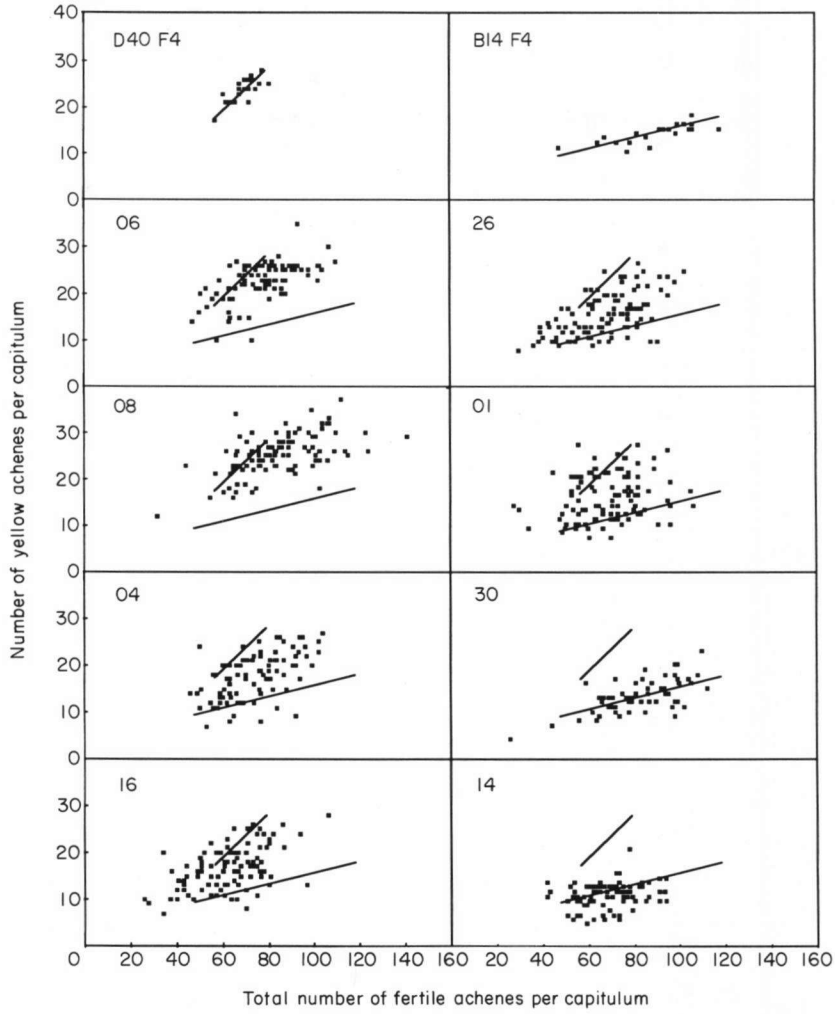
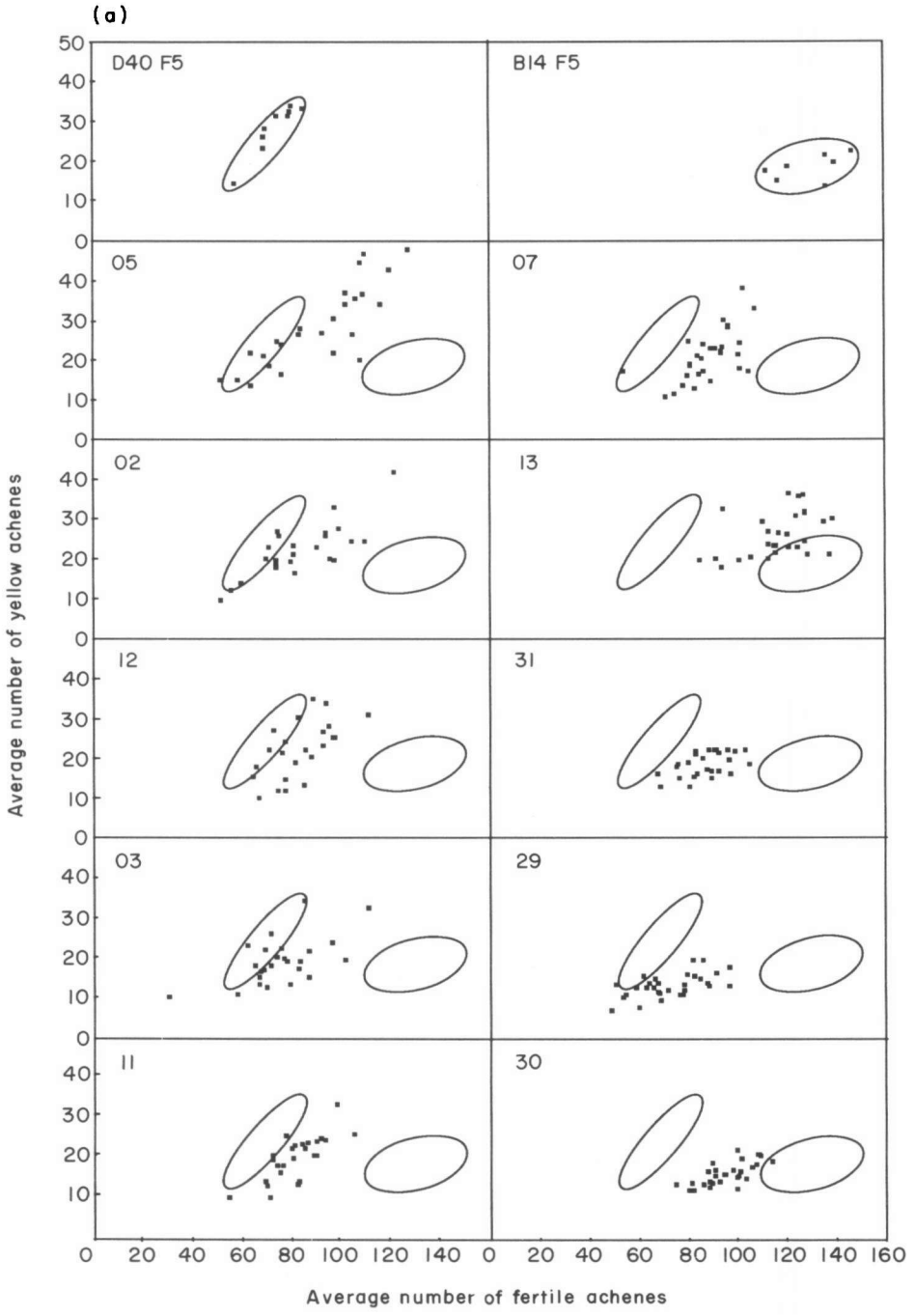


Fig. 4. Numbers of yellow achenes of F3 lines raised in 1986/1987 against numbers of fertile achenes in individual capitula. Regression lines for the parental strains (top panels: homozygotes for the main gene) are superimposed on the F3 data. Line numbers refer to the F2 parent plant (see Fig. 1).

coefficients of determination (r^2) are high in both years. The difference between the regression slopes reflects the year-to-year environmental variation. In order to relate variation in capitulum size and the proportion of yellow achenes, the results of F3 and F4 lines were analysed by plotting numbers of yellow achenes against the total number of achenes. To show the total possible range of variation, including plasticity, we set out the individual values of all three heads per plant of the F3 lines of 1986/1987 in Fig. 4. The regression lines of the homozygous control strains (top panels) represent the phenotypes corresponding to the homozygous parental genotypes of the main gene in their own genetic background.

Figure 5a shows the same data for the F3 lines raised in 1987/1988, based on average values per plant. Considerable between-plant plastic variation in capitulum size remains,


(a)
D40 F5
B14 F5
05
07
02
13
12
31
03
29
11
30
Average number of yellow achenes
Average number of fertile achenes
0 20 40 60 80 100 120 140
0 20 40 60 80 100 120 140 160
0 10 20 30 40 50

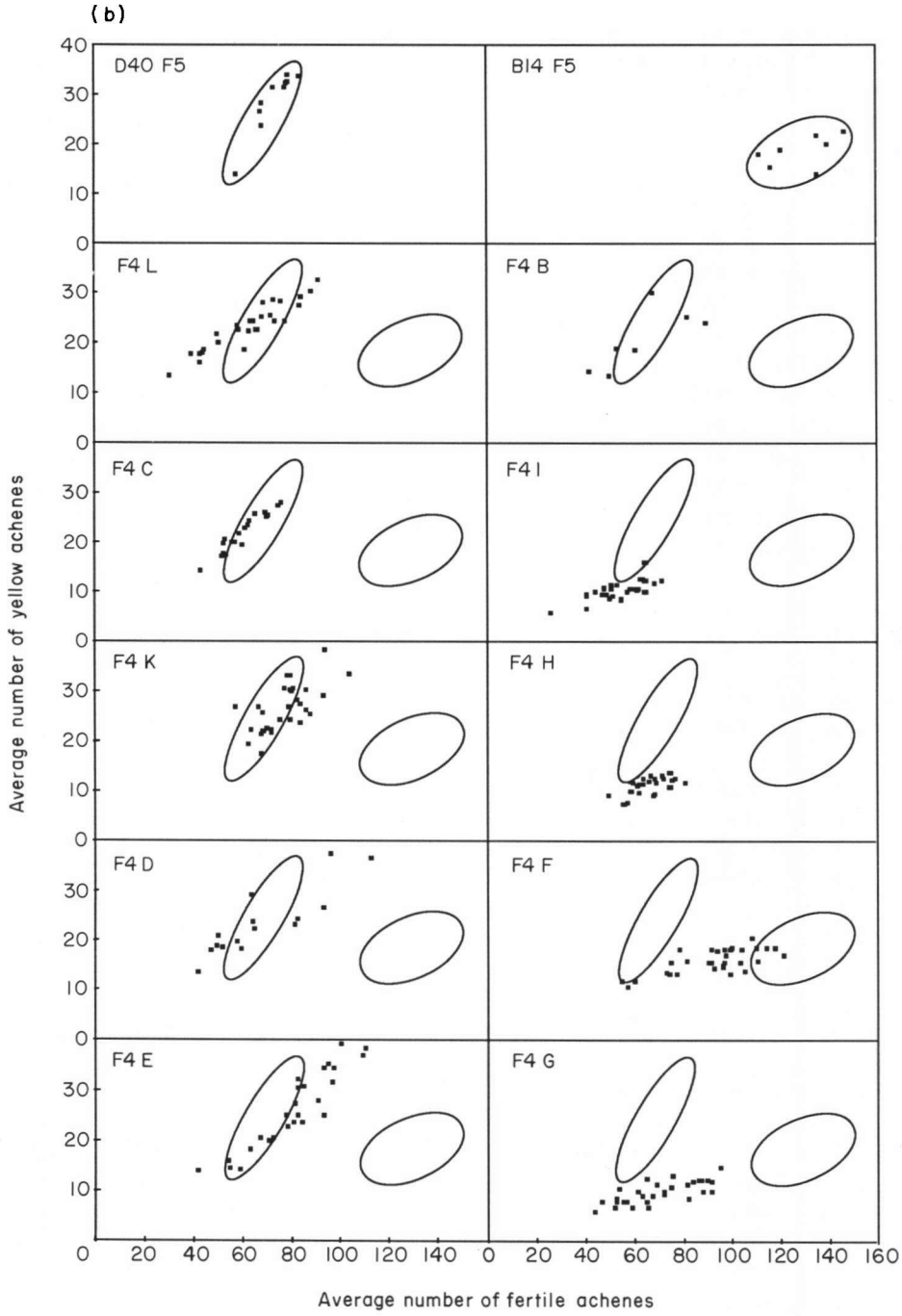


Fig. 5. Numbers of yellow achenes against numbers of fertile achenes. Parental control ranges (plastic variation in the homozygotes: top panels) are superimposed on the offspring segregations. (a) F3 lines raised in 1987/1988. Line numbers refer to the F2 parent plant (see Fig. 1). (b) F4 lines raised in 1987/1988.

as shown by the parental controls on top. The figure also illustrates clearly that F2 plants 31, 29 and 30 were homozygous for the high lf-value (low proportion of yellow achenes) allele from B14, while the phenotype for the total number of achenes strongly suggests that this character in these three plants is mainly determined by alleles from the D40 parent.

For the purpose of further characterizing and eventually mapping the gene responsible for the proportion of yellow achenes we have tried to isolate homozygous genotypes for this gene in various genetic backgrounds (recombinant inbreds) in the F4. In Fig. 5b, F4 lines H and I, for instance, show how the low proportion of yellow achenes from B14 has been recombined with the smaller capitulum size of D40. The figure also shows that further genetic factors besides those affecting capitulum size influence the proportion of yellow achenes. In F4 lines L to B, the D40 genotype for the proportion of yellow achenes has been recovered in heads of the same size as the D40 parent, yet the relationship between the number of yellow and the total number of achenes obviously differs from that in the inbred D40 parent. This effect corresponds to the observation that the low parental lf for the proportion of yellow achenes was not recovered in the F2 (Fig. 1). In contrast, the slopes for the four homozygous B14-like F3 lines and the four homozygous B14-like F4 lines are not significantly different from the slope of the B14 regression line. There is, therefore, a noticeable influence of the genetic background only on the expression of the D40 (high proportion of yellows) genotype while the expression of the B14 (low proportion) genotype is virtually independent of the genetic background.

DISCUSSION

Integrated analyses of genetic and developmental processes causing species or population differences in the capitulum structure of Compositae have rarely been undertaken. Many studies have been done either on the genetic basis of varying morphological characters (Richards 1975; Fick 1976; Mosjidis 1982) or from a developmental point of view (Popham & Chan 1952; Palmer & Marc 1982; Hilger & Reese 1983; Leins & Erbar 1987). However, integrated studies can be useful in understanding some of the evolutionary processes.

The radial differentiation gradient across the flowering head of composites is an extremely suitable model for such experiments because each floret and achene can be considered as a bioassay for the proposed morphogen gradient (Bachmann 1983). We explain the different proportions of morphologically distinguishable kinds of fruits on the same mature capitulum as differences in response thresholds of the genes that determine the characters to concentration differences of some biochemical substance, high around the rim and decreasing towards the centre of the head. Against the background of an equal proportion of hairy achenes in both parental strains and the hybrid, the genetic variation for the extent of the yellow fruit colour found here may be due to allelic differences in the response thresholds to a constant morphogen gradient. Even without any change in the relevant structural gene, genetic variation in the response threshold to the morphogen and genetic variation in morphogen concentration can explain the full range of quantitative variation from the expression of 'peripheral' characters all over the capitulum to their complete absence. Gottlieb & Ford (1987) explained the presence or absence of ray florets and their associated involucral bracts on the capitula of different species of *Layia* as the result of small mutations in genes that control, for example, time and rate of cell divisions in undifferentiated primordia, so that some primordia are retarded in development, are not subjected to the same biochemical or biophysical conditions and differentiate into

morphologically different organs, even though they possess the same genetical information. The authors reject the involvement of a morphogen on the basis of the fact that the receptacle volumes are equal in both species. As it is virtually impossible to distinguish temporal and spatial effects during capitulum development on the basis of adult morphology, the different explanations for similar differentiation gradients may eventually converge. Surgical manipulation is the most direct physiological approach to obtain information on tissue interactions during organ determination in a developing capitulum. In the large capitulum buds of sunflowers incisions can be made in very early bud stages when receptacle tissue has not yet been differentiated. Hernandez & Palmer (1988) reported that a cylindrical 1-mm diameter plug, isolated from adjacent tissue on a young sunflower capitulum, differentiated in a few days into a complete, functional capitulum, surrounded by involucral bracts, lifted by a stem-like structure somewhat above the original head. The disc florets on the newly formed head were geometrically arranged into a number of contact parastichies. According to the authors the formation of the three types of organs can be explained if the organ initiation is a natural property of receptacle tissue, released and controlled by the receptacle rim. It is conceivable that this stimulus corresponds to our proposed morphogen that operates from the rim towards the centre.

Morphogens have also been found in animal cells (Thaller & Eichele 1987; Driever *et al.* 1989). Recently, a diffusible plant morphogen has been demonstrated that causes epidermal cells of *Catharanthus roseus* (Apocynaceae) to redifferentiate into parenchyma (Siegel & Verbeke 1989).

As the regulatory processes leading to the differentiation gradient across a composite capitulum take place in tiny capitulum buds (Wardlaw 1963; Steeves *et al.* 1969) possibilities for direct physiological experimentation are generally limited. The data presented here should help to provide access to the physiological mechanism starting with the relevant genes. There are many advantages in localizing genes in working with recombinant inbred lines (reviewed in Bailey, 1981) and recombinant inbred lines in plants have already been used for mapping genes in *Pisum* (Domoney *et al.* 1986) and in maize (Burr *et al.* 1988), for plant breeding (Brim 1966) and for estimations of components of variance (Jinks 1981). We are now preparing a restriction fragment length polymorphism linkage map of several *Microseris* species to try to map the genes involved in the development of morphologically different kind of fruits on the capitula and the determination of their proportions.

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REFERENCES

Bachmann, K. (1983): Evolutionary genetics and the genetic control of morphogenesis in flowering plants. *Evol. Biol.* **16:** 157–208.

—, & Chambers, K.L. (1981): Genes regulating the appearance of two kinds of fruit in *Microseris* strain B87 (Asteraceae: Compositae). *Experientia* **37:** 29–31.

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

Bailey, D.W. (1981): Recombinant inbred strains and bilineal congenic strains. In: Foster, H.J., Small, J.D. and Fox, J.G. (eds.): *The Mouse in Biomedical Research.* **1:** 223–239.

Brim, C.A. (1966): A modified pedigree method of selection in soybeans. *Crop. Sci.* **6:** 220.

Burr, B., Burr, F.A., Thompson, K.H., Albertson, M.C. & Stuber, C.W. (1988): Gene mapping with recombinant inbreds in maize. *Genetics* **118:** 519–526.

Domoney, C., Ellis, T.H.N. & Davies, D.R. (1986): Organization and mapping of legumin genes in *Pisum*. *Mol. Gen. Genet.* **202:** 280–285.

Driever, W., Thomas, G. & Nüsslein-Volhard, C. (1989): Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the bicoid morphogen. *Nature* **340:** 363–367.

Fick, G.N. (1976): Genetics of floral color and morphology in sunflowers. *J. Hered.* **67:** 227–230.

Froebe, H.A. (1964): Die Blütenstände der Saniculoideen (Umbelliferae). *Beitr. Biol. Pflanzen* **40:** 325–388.

Gottlieb, L.D. & Ford, V.S. (1987): Genetic and developmental studies of the absence of ray florets in *Layia discoidea*. In: Thomas, H. and Grierson, D. (eds): *Developmental Mutants in Higher Plants*. 1–17. Cambridge University Press, Cambridge.

Hernandez, L.F. & Palmer, J.H. (1988): Regeneration of the sunflower capitulum after cylindrical wounding of the receptacle. *Am. J. Bot.* **75:** 1253–1261.

Hilger, H.H. & Reese, H. (1983): Ontogenie der Strahlblüten und der heterokarpen Achänen von *Calendula arvensis* (Asteraceae). *Beitr. Biol. Pflanzen* **58:** 123–147.

Jean, R.V. (1984): *Mathematical Approach to Pattern and Form in Plant Growth*. John Wiley & Sons, New York.

Jinks, J.L. (1981): The genetic framework of plant breeding. *Phil. Trans. R. Soc. Lond. B.* **292:** 407–419.

Leins, P. & Erbar, C. (1987): Studien zur Blütenentwicklung an Compositen. *Bot. Jahrb. Syst.* **108:** 381–401.

Mauthe, S., Bachmann, K., Chambers, K.L. & Price, H.J. (1984): Independent responses of two fruit characters to developmental regulation in *Microseris douglasii*. (Asteraceae, Lactuceae) *Experimentia* **40:** 1280–1281.

Mosjidis, J.A. (1982): Inheritance of color in the pericarp and corolla of the disc florets in sunflower. *J. Hered.* **73:** 461–464.

Palmer, J.H. & Marc, J. (1982): Wound-induced initiation of involucral bracts and florets in the developing sunflower inflorescence. *Plant Cell Physiol.* **23:** 1401–1409.

Pomplitz, R. (1956): Die Hetermorphie der Früchte von *Calendula arvensis* unter besonderer Berücksichtigung der Stellungs- und Zahlenverhältnisse. *Beitr. Biol. Pflanzen* **32:** 331–369.

Popham, R.A. & Chan, A.P. (1952): Origin and development of the receptacle of *Chrysanthemum morifolium*. *Am. J. Bot.* **39:** 329–339.

Richards, A.J. (1975): The inheritance and behaviour of the rayed gene complex in *Senecio vulgaris*. *Heredity* **34:** 95–104.

Rutishauser, R. (1981): Blattstellung und Sproßenentwicklung bei Blütenpflanzen unter besonderer Berücksichtigung der Nelkengewächse (Caryophylaceen s.l.). *Diss. Botanicae* **62:** 1–127.

Siegel, B.A. & Verbeke, J.A. (1989): Diffusible factors essential for epidermal cell redifferentiation in *Catharanthus roseus*. *Science* **144:** 580–582.

Steeves, T.A., Hicks, M.A., Naylor, J.M. & Rennie, P. (1969): Analytical studies on the shoot apex of *Helianthus annuus*. *Can. J. Bot.* **47:** 1367–1375.

Thaller, C. & Eichele, G. (1987): Identification and spatial distribution of retinoids in the developing chick limb bud. *Nature* **327:** 625–628.

Wardlaw, C.W. (1963): Experimental investigations of floral morphogenesis in *Petasites hybridus*. *Nature* **198:** 560–561.

Weberling, F. (1981): *Morphologie der Blüten und der Blütenstände*. Verlag, Stuttgart.