Heredity **64** (1990) 29-44

Received 23 May 1989

Genetic studies of floral evolution in *Layia*

V. S. Ford and
L. D. Gottlieb

Department of Genetics, University of California, Davis, CA 95616.

***Layia glandulosa* (Compositae) and *L. discoidea* are self-incompatible annual plants native to California which are completely interfertile and appear to be related as progenitor and recent derivative. *L. glandulosa* has sunflower-like heads (capitula) with showy female rays, each subtended by an involucral bract which enfolds the ovary. *L. discoidea* lacks both rays and enfolding bracts. We describe the results of a breeding programme to identify specific genes that control these and associated morphological traits. The differences in capitulum type are governed primarily by two genes, partially confirming the conclusions of Clausen, Keck and Hiesey (1947). Recombination of these genes produced a novel phenotype with "gibbous" florets in place of rays. Gibbous florets have aspects of both ray and disk florets as well as unique traits. They are fertile and consistent in expression, demonstrating that new combinations of developmental processes may be assimilated without evident adverse effects. Another recombinant genotype confers on ray florets traits such as ovary pubescence and pappus, normally found only on disk florets. Despite the absence of ray florets, *L. discoidea* has a polymorphism that affects ray presence/absence and additional genes modifying ray floret number, size, shape and colour. Thus, differences in floral morphology between the species depend on a complex assemblage of genes with significant and specific morphological consequences.**

INTRODUCTION

Plants are particularly appropriate for analysis of the genetic and developmental bases of morphological differences because related species are often interfertile, and in many cases discrete morphological differences appear to be governed by few genes (Gottlieb, 1984). Isolation of the effects of specific genes is an essential prerequisite to developmental genetic analysis and also can be used to initiate ecological studies testing the adaptive significance of particular allele substitutions. In this paper we describe the breeding programme we have started to isolate specific genetic components of a change in morphology that characterizes two species.

The genus *Layia* (Compositae, tribe Heliantheae, subtribe Madiinae) comprises fifteen species of Californian spring annual plants. Like other sunflower-type plants, most *Layia* species have floral heads (capitula) consisting of a swollen stem apex, termed a receptacle, on which are mounted an outermost ring of involucral bracts (also called phyllaries), then showy female rays or ray florets, and a central group of disk florets, sometimes associated with small receptacle bracts (figs 1A and 1B). In most Layias, each involucral bract is clasped or folded longitudinally around the ovary of an adjacent ray (fig. 1C), and there is a ring of receptacle bracts between the ray and disk florets (figs 1B and 1D). However, one species, *L. discoidea* Keck (a serpentine endemic found only in a small area in central California), lacks rays, its involucral bracts are not clasping, and it does not have an inner ring of receptacle bracts (figs 1E and 1F). The heads of *L. discoidea* look much like radiate heads with the rays and involucral bracts removed and the remaining ring of receptacle bracts in the position of an involucre (fig. 1D). *L. discoidea* is fully interfertile with a widespread species, *L. glandulosa* (Hook.) Hook. & Arn., which has large white (subsp. *glandulosa*) or yellow (subsp. *lutea*) rays. High genetic identity, determined by electrophoretic comparison of isozymes, confirms the two species are closely related (Gottlieb, Warwick and Ford, 1985). The direction of evolutionary change is clear, in this case, since the limited distribution, specialized serpentine habitat, and unusual floral morphology show that *L. discoidea* is the derived species.

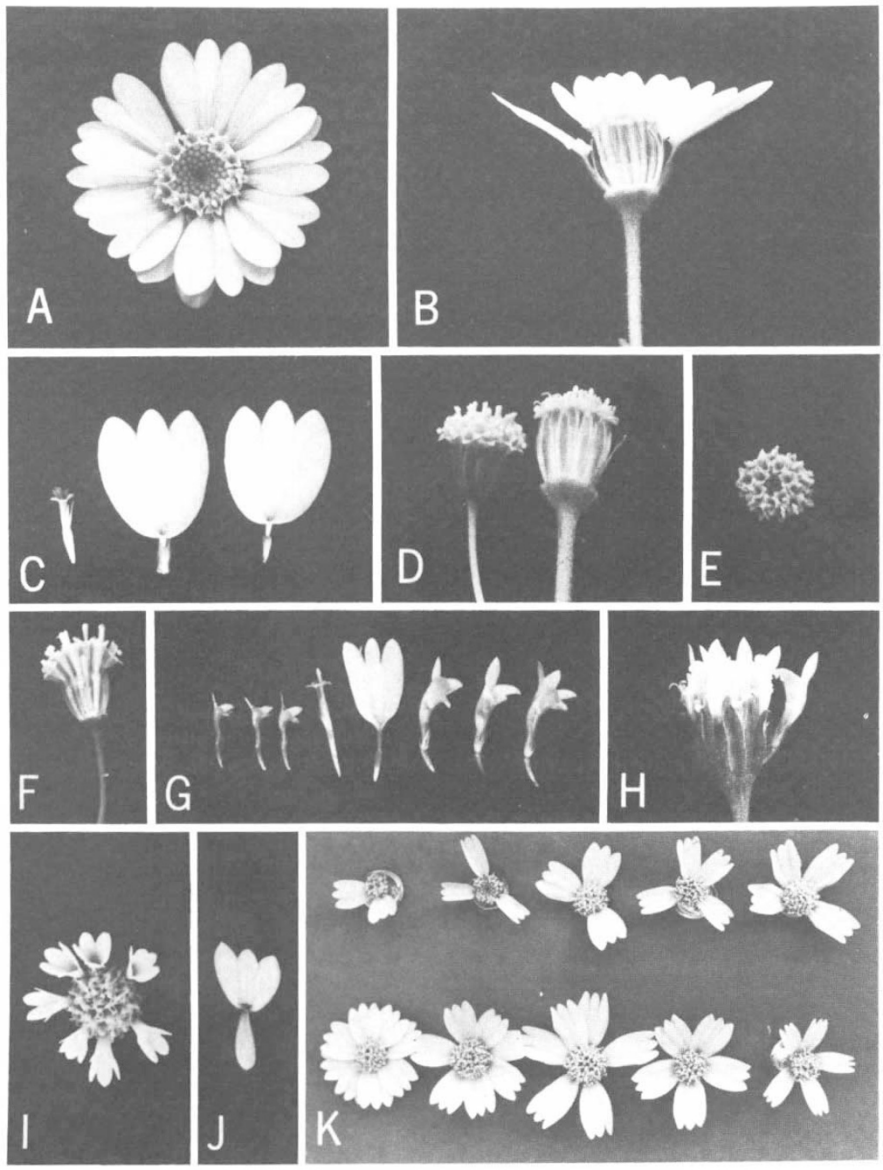

Figure 1 Capitula and florets of *L. discoidea*, *L. glandulosa* and hybrids. A. *L. glandulosa* subsp. *glandulosa*. B. Subsp. *glandulosa*, cut to expose disk florets and receptacle bracts. C. Subsp. *glandulosa* disk floret (left), rays with (centre) and without (right) clasping involucral bracts. D. (left) *L. discoidea* and (right) subsp. *glandulosa* with rays and involucral bracts removed to expose receptacle bracts. E. *L. discoidea*. F. *L. discoidea*, cut to expose disk florets. G. (left to right) Three gibbous florets from 8BR (genetic background 94 per cent from *L. discoidea*, 6 per cent from subsp. *lutea*), disk and ray florets from subsp. *lutea*, three gibbous florets from 8BN (background 44 per cent from *L. discoidea*, 56 per cent from subsp. *lutea*). H. Head of gibbous plant with gibbous floret pulled out to show orientation. I. Tubular rays. J. Opposite-lobe rays. K. heads from 6B (BC_2F_2, recurrent parent subsp. *lutea*), showing variation in number of rays per head and ray proportions.

The first experimental hybridization between the two species was described by Clausen, Keck and Hiesey (1947). A large F_2 progeny segregated 13:3, radiate versus discoid plants, suggesting that the absence of ray florets and enfolding involucral bracts in *L. discoidea* was governed by two genes. However the authors (1947, p. 119) acknowledged that it was difficult to classify the progeny because the parental differences segregated into a number of small steps. Variation in number and colour of rays suggested the influence of other genes from *L. discoidea*. The study was intriguing but incom-

plete since no replicate crosses were done, no progeny tests were made to confirm the assignment of genotypes to F_2 individuals, and no attempt was made to distinguish the individual effects of the two genes.

In taking up this system forty years later, our goal has been to discover what genetic differences in capitulum development account for the presence versus absence of ray florets. Our initial plan was to re-test the two-gene model, to make a comparison of head development in the two species, and to transfer, by backcrossing, the alleles characterizing each species into the genetic background of the other species for additional studies of their developmental effects. Results of the first two generations of backcrosses and scanning electron micrographs of early stages of head development were presented in Gottlieb and Ford (1987). Backcross segregation was consistent with the two-gene model. The development of *L. discoidea* heads showed no evidence of aborted ray florets or gaps in the regular helical progression of primordia. This observation and the occurrence of variant peripheral florets in some hybrid plants suggested to us that the genes governing the species difference may modify the development of the peripheral florets and bracts rather than suppressing or aborting them.

Here we describe our subsequent genetic analysis of ray floret presence versus absence in experimental progenies derived from hybrids between *L. discoidea* and both subspecies of *L. glandulosa*, including the evidence for an unexpected polymorphism in *L. discoidea* affecting floret type in hybrids. We also describe the genetic basis of a novel, stable phenotype ("gibbous florets") produced during the investigation but not known in nature, and some relationships between ray presence/absence and other attributes such as ray number, size, and ovary pubescence.

MATERIALS AND METHODS

Plants and crosses

Populations of *L. glandulosa* subsp. *glandulosa* (accession F8330, abbreviated GLA or G), *L. glandulosa* subsp. *lutea* (8319, LUT or L) and *L. discoidea* (8347, DIS or D, later re-collected as accession 8610) were collected and grown out as described in Gottlieb, Warwick and Ford (1985). The plants are diploid, $n = 8$. The subspecies of *L. glandulosa* are similar in head structure, but differ in ray colour (white versus yellow) and in quantitative traits (Gottlieb, Warwick and Ford, 1985; Ford and Gottlieb, 1989). Both species are strictly self-incompatible. Each progeny was made by crossing a single pair of individuals.

Plants grown from field-collected seed were used to make F_1, BC_1 and F_2 progenies. Parental seed stocks used for later backcrosses were obtained by cross-pollinating parent plants at random in the greenhouse. Progenies derived from *L. discoidea* and *L. glandulosa* subsp. *lutea* are diagrammed in fig. 2 and those derived from *L. discoidea* and *L. glandulosa* subsp. *glandulosa* in fig. 3. Seeds from reciprocal crosses between the same parent individuals were kept separate and, when possible, backcross progenies were grown from seed of the recurrent parent. However, no significant differences between reciprocal progenies (of either D×L or D×G types) were observed and, when both were used, the data were pooled.

Growing conditions

Achenes were germinated in petri dishes in water or, if germination followed less than four months after harvest, in 125 ppm gibberellic acid (GA_3). Plants grown in the spring were planted in 4 cm square "Cell-Paks" in February, transplanted to 10 cm pots about a month later, and maintained in an outdoor lath house under natural daylength. Plants grown in other seasons were planted and grown to maturity in 5 cm pots in controlled environment chambers. They were given alternating conditions of 12 h light (about 300 microeinsteins $m^{-2}\,s^{-1}$ at soil height) at 18°C and 12 h dark at 15°C for a month, then 16 h light at 21°C and 8 h dark at 18°C. Plants in "Cell-Paks" were fertilized weekly with half-strength Hoagland's solution and those in pots with "Osmocote" pellets.

Description of ray and disk florets and associated bracts

Disk florets have a bright yellow corolla tube with five small radially symmetrical lobes, a pappus of numerous bristles, five functional connate anthers forming a cylinder around the style, and densely pubescent ovaries. Ray florets are much larger, with a long three-lobed strap-shaped ligule surmounting a short corolla tube. The corolla may be white or yellow, depending on subspecies. In *L. glandulosa*, rays lack pappus, stamens, and ovary pubescence. There are no macroscopic differences in the style and stigma of ray and disk florets.

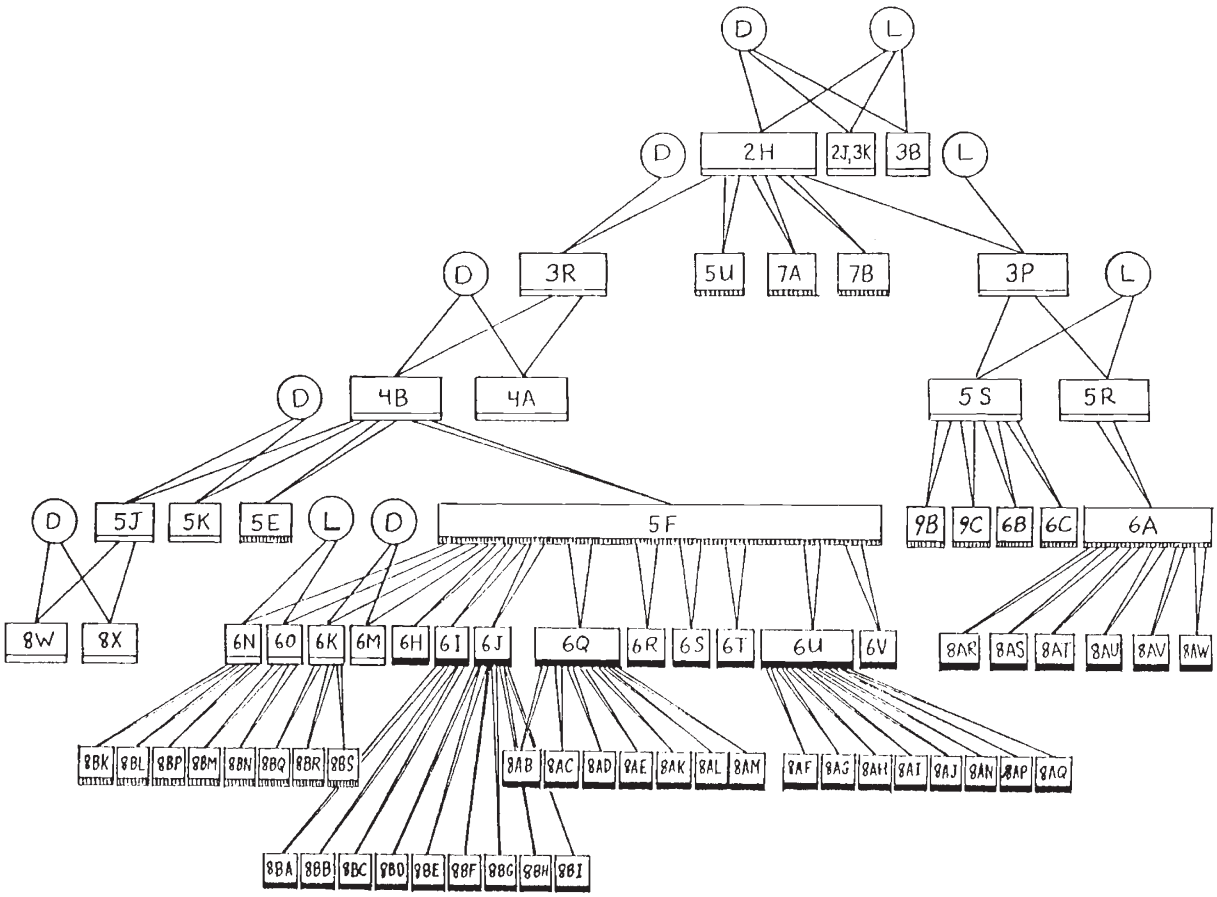

Figure 2 Progenies from hybrids of *L. discoidea* and *L. glandulosa* subsp. *lutea*. Circles denote species, rectangles marked with a white band denote F_1 and backcross progenies, shaded bands denote crosses of selected like individuals, black bands denote random crosses to test homozygosity. Prefix of progeny name indicates when progeny was grown (2: fall 1984; 3: spring 1985; 4: fall 1985; 5: spring 1986; 6: fall, 1986; 7: winter 1987; 8: spring 1987; 9: fall 1987).

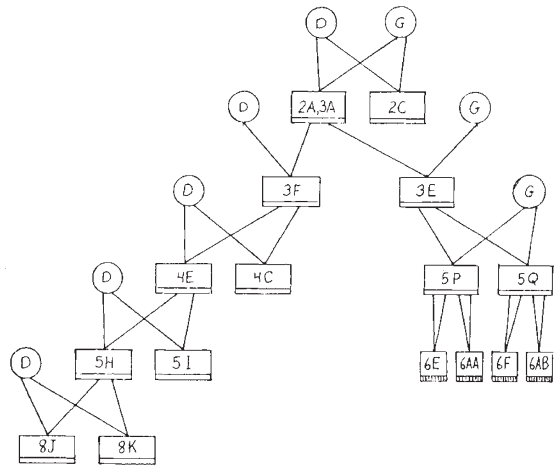

Figure 3 Progenies from hybrids of *L. discoidea* and *L. glandulosa* subsp. *glandulosa*. Symbols and prefixes as in fig. 2.

Ray corollas of hybrid plants are various shades of pale yellow. Most are shaped like those of *L. glandulosa*, but smaller, and may have some degree of ovary pubescence, pappus and/or rudimentary stamens, although never as much as disk florets. Ray corollas are occasionally asymmetrical, have varying numbers of lobes, or a trumpet-like tube instead of a ligule (fig. 1I). When extra lobes are present, they may be on the ligule or opposite to it (fig. 1J). Rays with abnormal shape usually have some ovary pubescence, pappus, or stamens as well. Disk florets of some hybrids may have distinct sectors of ray-like corolla tissue (indicated by colour and lobe shape), or may have only inconspicuously enlarged outer lobes. Such abnormal disk florets are always in a peripheral position, like rays. They are like normal disk florets with respect to ovary pubescence, pappus and anthers.

In *L. glandulosa*, only ray florets are paired with subtending clasping bracts. However, in both species, the outermost disk florets are subtended by bracts which are curved at most halfway around the ovary, and are shorter and more pointed than the clasping bracts. The inner disk florets lack subtending bracts. In hybrid plants, peripheral florets of all the types discussed above may be paired with flat, clasping, or intermediate bracts.

Characters scored

All plants were classified by capitulum type. Any floret with a ligule was considered a ray, and any plant with at least one ray was considered radiate. This strict criterion was adopted because some progenies had a continuum of radiate plants ranging from those with rays on every head to those with only one ray on one head. Some plants had peripheral florets which could not be classified as ray or disk florets. One type, which occurred consistently, we called a gibbous floret. It is described below (see Results).

The number of ray, gibbous or other peripheral florets was scored on the first four heads, *i.e.*, those terminating the main stem and each of the top three branches. These are usually the first capitula to reach anthesis. Relatively few plants had the same number of rays on every head but the range of numbers for each plant was limited, *e.g.*, a plant with eight rays on one head would be unlikely to have fewer than five on any other. Summary data are reported for the first head only since these data adequately characterize each group. In some groups, first heads averaged more rays than subsequent heads, but in other progenies the reverse was the case (data not shown). Selection for high or low ray number was based on the number on the first one or two heads.

Other characters scored in particular progenies included presence or absence of ray ovary pubescence, pappus and stamens, extent of involucral bract curvature, and ligule length and width on the first head (first three heads in spring 1985).

RESULTS

Evidence for two genes with large effects

For each subspecies of *L. glandulosa*, four generations of backcross progenies with *L. discoidea* as recurrent parent were grown (figs 2 and 3). In each case the backcross plants used as parents were selected for high ray number, usually 3–5 rays on the first head. Among the fourteen progenies there was a significant departure from homogeneity: nine segregated 1:3 for rays present versus absent, but the other five were not consistent with this model and showed diverse ratios (table 1). Four backcrosses (two from subsp. *lutea* and two from subsp. *glandulosa*) had significantly more than $\frac{1}{4}$ radiate plants; of these, two segregated 3:5 for rays present versus absent, one 1:1, and one fitted either model equally well (table 1). One progeny (5K) fitted a 1:7 model, one of several possible models involving three or more genes (table 1).

The 1:3 ratio suggests ray presence versus absence is largely governed by two independent loci, here designated R/r and G/g. However, the heterogeneity of the backcross ratios suggests polymorphism in the recurrent parent, *L. discoidea*. The simplest model consistent with the excess of radiate plants in some progenies is that the allele characteristic of *L. glandulosa* (or a similar allele that confers ray presence) at one of these loci is present in *L. discoidea*. The polymorphic locus is arbitrarily assigned as G/g.

Since ray presence is dominant (F_1 plants were radiate, table 1), genotype *RR GG* was assigned to *L. glandulosa*, while *L. discoidea* has alleles *r*, *g*, and *G*. Thus $R-G-$ backcross plants have rays while *Rr gg*, *rr Gg*, and *rr gg* plants do not. Since all *L. discoidea* plants are discoid, the model further requires that *rr* plants lack rays, regardless of the genotype at G/g.

This model predicts that F_1 plants can be *Rr Gg* or *Rr GG*, depending on the genotype of the *L. discoidea* parent. A radiate *Rr Gg* plant crossed to a discoid plant of genotype *rr gg*, *rr Gg*, or *rr GG* would yield progeny segregating 1:3, 3:5, or 1:1, respectively, for rays present versus absent. A radiate *Rr GG* plant crossed to any discoid plant would yield progeny segregating 1:1. Thus, the nine backcross progenies segregating 1:3 presumably resulted from crosses of type *Rr Gg* (from the F_1 or BC_n) × *rr gg* (from *L. discoidea*), two progenies (4A, 5H in table 1) segregating 3:5 from crosses of type *Rr Gg* × *rr Gg* and one (8W in table 1) segregating 1:1 from a cross of type *Rr Gg* × *rr GG*. (The BC_3 parent of 8W was a radiate plant from progeny 5J (fig. 2) which segregated 1:3 (table 1), so it must have been *Rr Gg*.) The genotypes of the parents of 8J are ambiguous.

If this assessment is correct, allele *G* is infrequent in the *L. discoidea* seed stock, since it appears in only three of the twelve recurrent parents with identifiable genotypes (9 *rr gg*, 2 *rr Gg*, 1 *rr GG*).

Three F_2 progenies from a single F_1 between *L. discoidea* and subsp. *lutea* segregated 3:1 for

Table 1 Number of radiate and discoid plants, and number of rays on first head, in *L. glandulosa*, F_1, F_2, and backcross progenies to *L. discoidea*. F_1 progenies 3K and 3A are the same as 2J and 2A, respectively, but grown at different times. D, L, G denote *L. discoidea*, *L. glandulosa* subsp. *lutea* and *L. glandulosa* subsp. *glandulosa*, respectively. $BC_n(Y, Z)$ denotes *n*th generation backcross with recurrent parent from Y, non-recurrent parent from Z. Statistics for ray floret number are based on radiate plants only

Group name		Radiate	Discoid	Total	χ^2 for genetic models				Number of ray florets	
									Mean	CV
L. glandulosa										
3L	subsp. *lutea*	32		32					8·6	13
4L	subsp. *lutea*	35		35					7·9	6
3G	subsp. *glandulosa*	36		36					8·9	16
6G	subsp. *glandulosa*	17		17					8·6	15
F. progenies										
2H	D×L	24		24					3·5	40
2J	D×L	19		19					5·8	15
2A	D×G	24		24					5·2	20
2C	D×G	12		12					5·7	16
3K	D×L	22		22					5·3	18
3B	D×L	26		26					5·0	21
3A	D×G	41		41					4·8	18
						3:1				
F_2 *progenies*										
5U	D×L	164	54	218		0·01				
7A	D×L	186	65	251		0·11				
7B	D×L	229	80	309		0·13				
Backcrosses to L. discoidea					1:7	1:3	3:5	1:1		
3R	BC_1(D, L)	24	72	96		0·00			2·4	44
3F	BC_1(D, G)	22	45	67		2·19			3·0	42
4A	BC_2(D, L)	39	57	96		12·50***	0·40	3·38	2·8	43
4B	BC_2(D, L)	21	60	81		0·04			2·4	52
4C	BC_2(D, G)	24	58	82		0·80			2·9	39
4E	BC_2(D, G)	21	58	79		0·11			3·2	38
5J	BC_3(D, L)	21	86	107		1·65			2·0	47
5K	BC_3(D, L)	12	94	106	0·13	10·58**			1·0	100
5H	BC_3(D, G)	35	64	99		5·66*	0·19	8·49**	1·5	62
5I	BC_3(D, G)	31	102	133		0·20			2·2	38
8W	BC_4(D, L)	50	46	96[1]		37·56***	8·71**	0·17	2·5	48
8X	BC_4(D, L)	22	94	116		2·25			2·5	42
8J	BC_4(D, G)	46	59	105		19·81***	1·78	1·61	2·8	36
8K	BC_4(D, G)	26	75	101		0·03			2·8	58
Total backcross χ^2						83·61				
Pooled backcross data		394	980	1374		9·90**				
Heterogeneity for 1:3 genetic model						73·71***				
Heterogeneity for 394:980 stastistical model						67·57***				

[1] 10 plants not clearly identifiable as radiate or discoid were excluded

* $P < 0·05$.
** $P < 0·01$.
*** $P < 0·001$.

rays present versus absent (table 1). This suggests the *L. discoidea* parent was *rr Gg* and thus half the F_1 plants *Rr GG*. Either an *Rr GG* × *Rr GG* or *Rr GG* × *Rr Gg* F_2 cross would yield radiate and non-radiate plants in 3:1 proportion.

A third locus appears necessary to account for the 1:7 segregation in backcross 5K (table 1). This progeny was unique both in having a low proportion of radiate plants and in having the fewest rays per head (table 1). This suggests the involvement of a gene which both reduces ray number and suppresses ray presence entirely on some *R*− *G*− plants.

Polymorphism test: Discoid plants grown from field-collected seed (accession 8610, same site as 8347) were crossed with radiate plants obtained

Table 2 Heterogeneity of progeny segregations for two discoid plants crossed to the same radiate plant and vice versa (* $P<0{\cdot}05$). Because of incompatibility and/or poor seed set, only 12 pairs of crosses were complete and these are identified as Progeny Type A. Type B identifies two sets with progenies from one radiate × two discoid plants and a second radiate × one discoid plant. Type C identifies two sets with progenies from one radiate × two discoid plants. Type D identifies one set with progenies from one radiate × three discoid plants. Type E identifies four sets with progenies from one radiate × one discoid plant. Progeny not identifiable as radiate or discoid are also reported (X) and used in computing heterogeneity. Radiate parents marked 'P' had normal rays, 3–6 rays on first head

Progeny type	Parent names		Number of progeny			Heterogeneity χ^2		Most likely model
	Discoid	Radiate	Radiate	X	Discoid	Discoid	Radiate	R:D
A	9DD6	9AC40 P	26	6	74	12·17*	5·35	1:3
	9DD51	9AC40 P	45	12	49		14·48*	2:1
	9DD6	9AD12	4	0	31	5·19		1:7
	9DD51	9AD12	32	7	93			1:3
A	9DD20	9AD9 P	41	3	62	1·20	11·95*	3:5
	9DD22	9AD9 P	53	6	65		4·77	1:1
	9DD20	9AC18	74	13	53	0·30		1:1
	9DD22	9AC18	72	14	59			1:1
A	9DD43	9AA20	15	0	105	11·20*	1·26	<1:3*
	9DD44	9AA20	0	0	83		8·13*	<1:3*
	9DD43	9AA26	14	0	152	0·05		<1:3*
	9DD44	9AA26	5	0	48			<1:3*
A	9DD37	9AD5 P	10	0	137	35·08*	0·87	<1:3*
	9DD23	9AD5 P	68	0	135		4·78*	3:5
	9DD37	9AC8	4	0	96	12·13*		<1:3*
	9DD23	9AC8	17	0	66			1:3
A	9DD33	9AC17 P	79	0	104	23·86*	13·26*	3:5
	9DD35	9AC17 P	38	0	154		14·07*	1:3
	9DD33	9AB15 P	49	3	32	13·01*		1:1
	9DD35	9AB15 P	27	4	56			3:5
A	9DD12	9AD4 P	50	23	77	2·75	2·69	3:5
	9DD26	9AD4 P	68	17	89		3·10	3:5
	9DD12	9AD25 P	64	23	126	0·19		3:5
	9DD26	9AD25 P	61	24	115			3:5
B	9DD48	9AD2	6	0	55	2·82	0·00	<1:3*
	9DD2	9AD2	16	0	63			1:3
	9DD48	9AC6	15	4	140			<1:3*
B	9DD39	9AA1	30	0	94	0·55	1·73	1:3
	9DD42	9AA1	23	0	91			1:3
	9DD39	9AB6 P	39	4	84			3:5
C	9DD29	9AC27	28	0	90	1·20		1:3
	9DD25	9AC27	20	0	92			1:7
C	9DD19	9AD10 P	24	0	63	9·56*		1:3
	9DD8	9AD10 P	17	0	129			<1:3*
D	9DD16	9AA28 P	27	0	99	5·37*		1:3
	9DD46	9AA28 P	16	0	86			<1:3*
	9DD3	9AA28 P	33	0	82			1:3
E	9DD4	9AC13 P	49	0	77			3:5
	9DD30	9AA11 P	32	5	106			1:3
	9DD47	9AD8	9	0	150			<1:3*
	9DD1	9AC23	10	0	88			<1:3*
Replicates of progenies grown previously (*Table* 1)								
repeat-4A			65	0	58			1:1
repeat-5H			27	0	53			3:5
repeat-8W			61	13	66			1:1
repeat-8J			51	0	120			1:3

by re-growing second generation backcross progeny 4B (re-grown as 9A). Since that progeny segregated $\frac{1}{4}$ radiate plants (table 1), the radiate plants were putatively *Rr Gg*. Each radiate plant was crossed with two discoid plants and vice versa. Data are reported for 41 progenies (table 2) because the crossing design was incomplete since some pairs of plants proved incompatible and some progenies had too few seed. Of 16 pairs and one trio of discoid plants crossed to the same

radiate plant, eight yielded heterogeneous progenies, confirming polymorphism in *L. discoidea* for genes affecting ray presence.

Of fourteen pairs of radiate plants crossed to the same discoid plant, six yielded heterogeneous progenies, indicating genetic variability among the group of putative *Rr Gg* plants. Among the 41 progenies, 12 had significantly less than $\frac{1}{4}$ radiate plants, confirming the influence of another gene or genes affecting ray presence. Like 5K, these progenies generally fit a 1:7 (3 gene) or 1:15 (4 gene) model, but the sample size was not sufficient to exclude alternative possibilities (*e.g.*, 9DD6 × 9AD12 or 9DD25 × 9AC27). Also like 5K, these progenies had low numbers of rays per head (data not shown), suggesting the influence of genes with effects on both ray presence and ray number. Of the 12 radiate parents with well-formed rays and relatively high ray numbers (P in table 2), only three produced a progeny with less than $\frac{1}{4}$ radiate plants, while seven out of eleven radiate parents with fewer rays produced such a progeny. This strengthened the connection between ray presence and number, and showed that selection for high ray number was important in maintaining the high proportions of radiate plants in the four generation backcrossing program (table 1).

The four backcrosses which originally showed significantly more than $\frac{1}{4}$ radiate plants (see table 1) were re-grown; three again had a significant excess of radiate plants (table 2).

Gibbous florets

In order to identify the phenotype of homozygous recombinant *RR gg* plants and to advance the goal of producing *RR GG* plants on the *L. discoidea* background, compatible pairs of radiate (*Rr Gg*) plants from BC_2 progeny 4B, of which subsp. *lutea* was the non-recurrent parent, were crossed to make the BC_2F_2 progenies 5E and 5F (fig. 2). These progenies were expected to include plants of all *R/r G/g* genotypes on an 87 per cent *L. discoidea* background. In each progeny, radiate plants comprised $\frac{9}{16}$, the proportion expected for genotypes *R− G−*, and discoid plants $\frac{5}{16}$ (table 3).

The remaining $\frac{2}{16}$ of each progeny (table 3) exhibited novel peripheral florets designated "gibbous florets" (figs. 1G and 1H). These were larger than disk florets but smaller than rays and had the pale yellow colour of hybrid rays rather than the bright yellow of disk florets. They had a bilaterally symmetrical tubular corolla with three large outer lobes and two small inner lobes. The outer side of the corolla tube was longer than the inner side and bulged out at the base (hence the term "gibbous") and curved back in at the top. Characteristic folds of corolla tissue were present within the bulged region. Gibbous florets had dense ovary pubescence and pappus. They also had anthers which in some cases released well-formed pollen. (Pollen fertility has not yet been tested.) They set seed when pollinated. Gibbous florets thus have aspects of both rays (size, colour, bilateral symmetry) and disk florets (five corolla lobes, no ligule, ovary pubescence, pappus, anthers) as well as unique traits (corolla bulge, tissue folds) (figs. 1G and 1H).

Of the $\frac{2}{16}$, about half had strong expression of gibbous florets, *i.e.*, gibbous florets on all heads, with the number per head comparable to the number of rays per head on radiate plants (table 3). The other half, with weak expression, formed a heterogeneous group ranging from plants with large gibbous florets but only on early heads to plants with only a few slightly gibbous florets, barely distinguishable from disk florets.

We proposed that the strongly gibbous plants were *RR gg* and that the plants with weak expression, together with an equal number of discoid plants, were *Rr gg*, *i.e.*, that genotype *Rr gg* had about 50 per cent penetrance in progenies 5E, 5F. Thus, the discoid plants in these progenies comprised genotypes *rr G−* ($\frac{3}{16}$), *rr gg* ($\frac{1}{16}$), and half the *Rr gg* plants ($\frac{1}{16}$). That heterozygous gibbous plants had not been recognized in the backcrosses to *L. discoidea* was presumably due to reduced penetrance and expressivity: some slightly gibbous plants from 5E and 5F were not distinctly different from some plants scored as discoid (with distorted peripheral disk florets) in backcross progenies. Strongly gibbous plants would be expected in a true F_2 initiated by an *RR GG* × *rr gg* cross, but not if either F_1 parent was *Rr GG*, as was apparently the case described above.

To test the model, plants with strong expression of gibbous florets (putative *RR gg*) were selected from progeny 5F, three pairwise crosses were made, and two plants were crossed to subsp. *lutea* and two to *L. discoidea*. The paired gibbous crosses yielded uniformly gibbous progenies (6H, 6I, 6J in table 3), as did eight additional crosses within 6I and 6J (table 3), consistent with the homozygosity of strongly gibbous plants.

The crosses to subsp. *lutea* yielded uniformly radiate progenies (6N, 6O in table 3). Four F_2 progenies (8BK, 8BL, 8BM, 8BN in table 3) all segregated 3:1 radiate versus gibbous, and a fifth (8BP) had a slight excess of gibbous plants. The results confirm a single gene difference between homozygous gibbous plants and subsp. *lutea*.

Table 3 Number of radiate (R), gibbous (G), weakly expressed gibbous (WG) and discoid (D) plants in BC_2F_2 progenies (recurrent parent *L. discoidea*, non-recurrent parent subsp. *lutea*) and in progenies testing genetic model for gibbous florets. Also, means and coefficients of variation for number of ray and gibbous florets on first head of radiate (R) and gibbous (G) plants respectively

Progeny		Head type					Model		Number of rays		Number of gibbous	
		R	G	WG	D	Total			Mean	CV	Mean	CV
							R:G+WG:D	χ^2				
Progenies from crosses of radiate plants in 4B												
5E	BC_2F_2(D, L)	223	18	21	102	364	9:2:5	3·77	2·7	48	1·8	71
5F	BC_2F_2(D, L)	194	22	16	106	338	9:2:5	0·51	2·5	62	3·9	30
Progenies from crosses of gibbous plants in 5F												
6H			24			24					5·1	20
6I			27			27					4·6	29
6J			24			24					5·9	20
Progenies from crosses in 6I, 6J												
8BA			32			32						
8BB			41			41						
8BC			29			29						
8BD			36			36					7·2	12
8BE			28		8	36						
8BF			32			32					5·3	18
8BG			33			33					5·6	17
8BH			36			36					5·0	10
8BI			29			29					5·3	13
Progenies of gibbous plants × subsp. lutea												
6N	F_1	35				35			7·0	16		
6O	F_1	36				36			7·6	10		
							R:G	χ^2				
8BK	F_2	61	24			85	3:1	0·47	7·4	11	7·3	12
8BL	F_2	78	25			103	3:1	0·03	6·7	19	6·3	20
8BM	F_2	76	29			105	3:1	0·38	7·9	17	6·8	18
8BN	F_2	88	19			107	3:1	2·99	7·7	12	7·2	8
8BP	F_2	63	34			97	3:1	5·23*	6·9	18	6·6	18
Progenies of gibbous plants × L. discoidea												
6K	F_1			26	10	36	70% penetrance					
6M	F_1	13		8	14	35	1:1 radiate vs other			$\chi^2 = 2·31$		
							G:WG:D	χ^2				
8BQ	F_2		31	30	38	99	1:1·4:1·6	2·27				
8BR	F_2		26	33	48	107	1:1·4:1·6	1·18				
8BS	F_2		22	28	37	87	1:1·4:1·6	0·34				

Cross 6K to *L. discoidea* (table 3) yielded 70 per cent slightly gibbous plants and 30 per cent discoid. All three F_2 progenies (8BQ, 8BR, 8BS in table 3) recovered $\frac{1}{4}$ strongly gibbous plants and were consistent with a model of 70 per cent penetrance in heterozygotes, supporting a single gene difference between homozygous gibbous plants and the predominate homozygous genotype of *L. discoidea.*

Collectively, these data confirm the strongly gibbous plants as *RR gg* and further strengthen the case for *R*/*r* and *G*/*g* as major genes governing ray presence/absence. A parallel series of crosses between *L. discoidea* and subsp. *glandulosa* was attempted but showed severe inbreeding depression, so the phenotype of *RR gg* plants on a subsp. *glandulosa* background remains to be confirmed.

Cross 6M of a gibbous plant to *L. discoidea* (table 3) yielded radiate, weakly gibbous and discoid plants. Probably the *L. discoidea* parent of this cross was *rr Gg*, in which case a 1:1 segregation of radiate versus weakly gibbous and discoid would be expected.

Table 4 Progeny tests to determine genotypes of selected radiate plants in 5F, 6Q, 6U. Means and coefficients of variation for number of ray and gibbous florets on first heads

Progeny name		Results R	G	D	Model		χ^2	Deduced parental genotypes	Number of rays Mean	CV	Number of gibbous Mean	CV
Plants from 5F												
6Q		36						*RRGG RRGG*	5·4	19		
6R		22	14		R:G	3:1	3·70	*RRGg R – Gg*	6·1	18	5·0	33
6S		26	6	2	R:(G+D)	3:1	0·04	*RRGg R – Gg*	4·5	23	3·8	58
6T		28	3	4	R:(G+D)	3:1	0·47	*RRGg R – Gg*	5·0	31	5·3	11
6U		33						*RRGG RRGg*	5·8	20		
6V		19	8	5	R:G:D	9:2:5	6·56*	*RrGg RrGg*	4·7	26	5·3	26
Plants from 6Q *with high and low ray number*												
8AB	high	50						*RRGG RRGG*	6·4	20		
8AC	high	54						*RRGG RRGG*	6·0	20		
8AD	high	36						*RRGG RRGG*	6·5	18		
8AE	high	46						*RRGG RRGG*	6·0	20		
8AK	low	32						*RRGG RRGG*	5·3	19		
8AL	low	34						*RRGG RRGG*	5·1	25		
8AM	low	26						*RRGG RRGG*	5·0	18		
Plants from 6U *with high and low ray number*												
8AF	high	15	8		R:G	3:1	1·17	*RRGg RRGg*	6·1	19	5·4	34
8AG	high	37						*RRGG RRG–*	6·8	17		
8AH	high	32						*RRGG RRG–*	7·5	8		
8AI	high	33						*RRGG RRG–*	7·4	11		
8AJ	high	30	16		R:G	3:1	2·35	*RRGg RRGg*	6·7	18	5·9	26
8AN	low	25						*RRGG RRG–*	6·5	17		
8AP	low	24	4	2	R:(G+D)	3:1	0·40	*RRGg RRGg*	5·4	22	4·8	36
8AQ	low	30						*RRGG RRG–*	5·8	17		

Progeny tests in 5F

Nine-sixteenths of the plants in the BC_2F_2 progenies (5E, 5F) were phenotypically radiate, but only $\frac{1}{16}$ were expected to be homozygous (*RR GG*). To identity homozygotes and produce a true-breeding line, pairs of radiate plants from 5F were crossed and six progenies were grown out (6Q, 6R, 6S, 6T, 6U, 6V; fig. 2; table 4). Analysis of segregation ratios permitted identification of many of the genotypes of the twelve parents. progeny 6Q was uniformly radiate, and seven additional progenies from crosses within 6Q were all radiate (table 4), indicating both parents of 6Q were *RR GG*. 6U was uniformly radiate but not true-breeding: crossing within 6U yielded five all-radiate progenies and three progenies segregating 3:1 radiate versus gibbous (table 4). Thus the parents of 6U appear to have been *RR GG* and *RR Gg*. Progenies 6R, 6S, and 6T segregated 3:1 radiate versus gibbous (table 4) so one parent of each was *RR Gg* and the other parent *RR Gg* or *Rr Gg*. The parents of 6V were probably both *Rr Gg* although the segregation ratio does not quite fit the 9:2:5 model (table 4).

Since only one of 81 random crosses of radiate plants in 5F was expected to produce a true-breeding progeny, the fact that one of six attempts was successful suggests the selection criteria employed (discussed below) were informative.

Effect of G/g on ray ovary pubescence and stamens

Ray ovary pubescence, pappus and rudimentary stamens were first observed in F_1 hybrids, and assumed to be effects of the *L. discoidea* genetic background. All radiate plants in the BC_2F_2 progenies 5E, 5F were examined for these features and, when possible, similar plants were paired for progeny-testing. Ray ovary pubescence and/or rudimentary stamens proved to be absent in *GG* plants (both parents of 6Q, one parent of 6U), but present in *Gg* plants. Similarly, these features were absent in 6Q and the progenies derived therefrom (all *RR GG*), but present in about half the plants in 6U (half *RR Gg*, half *RR GG*). In 6U, three pairs of plants with ray ovary pubescence (parents of 8AF, 8AJ, 8AP) were shown to be *RR Gg*, since

their progenies were $\frac{1}{4}$ gibbous. Five crosses in which one or both parents lacked ovary pubescence (putative *RR GG*) yielded all-radiate progenies. Thus, the progeny tests indicated that ray ovary pubescence and rudimentary stamens are associated with the gibbous allele *g* or a linked gene.

Independent confirmation of this effect was provided by crosses of plants from 5S (fig. 2), a second generation backcross progeny with recurrent parent subsp. *lutea*. Four radiate plants with large, though non-functional, ray floret anthers were crossed and both progenies (9B, 9C) segregated 3:1, radiate versus gibbous (for 9B, 23:6, $\chi^2 = 0{\cdot}29$; for 9C, 38:16, $\chi^2 = 0{\cdot}62$), although the gibbous phenotype was somewhat different on this background. Thus, again, plants with rudimentary ray floret anthers proved to be *Gg*.

Layia discoidea genes modifying ray number

F_1 progenies averaged fewer rays per head than *L. glandulosa* (table 1). To determine whether this effect was a consequence of the *Rr Gg* genotype or of other genes from *L. discoidea*, BC_1 progenies 3R, 3F were compared with F_1 progenies 3K, 3B, and 3A, grown at the same time and under the same conditions. Radiate backcross plants were *Rr Gg*, as were at least half of each F_1 (the other F_1 plants were *Rr GG* if the *L. discoidea* parent was *rr Gg*). Therefore, in the absence of other genes affecting ray number, at least half the F_1 plants (those of genotype *Rr Gg*) should have been similar in ray number to the radiate backcross plants. But, 83/89 F_1 plants averaged at least three rays on the first four heads, while only 3/46 radiate backcross plants had that many. Thus, there was almost no overlap in ray number phenotype between the F_1 and BC_1 progenies. The difference is attributed to other *L. discoidea* genes reducing ray number.

The number of rays per head remained stable over four generations of backcrossing (table 1), presumably indicating the maintenance of alleles from the non-recurrent parent (*L. glandulosa*) by selection for high ray number.

In test progenies 6U and 6Q, both uniformly *RR*, plants with high and low ray number differed appreciably only on the first head, eight rays versus five, generally reverting to five rays on later heads. However, crosses of plants with high ray number yielded progenies with higher average ray number (on first heads) than crosses with low ray number (for 6U, $F = 38{\cdot}48$, $p < 0{\cdot}001$; for 6Q, $F = 50{\cdot}02$, $P < 0{\cdot}001$). Regression of offspring on mid-parent values for ray number for first heads provides a heritability estimate of 0·32 for 6U and 0·39 for 6Q, additional evidence for genetic variation in ray number independent of *R/r*.

Effects of R/r, G/g on ray number

The twelve progeny-tested radiate plants from 5F (table 4) were all selected for high ray number, five to eight rays per head, in the hope of increasing the odds of choosing *RR GG* plants. Among these, at least seven, possibly as many as ten, were *RR*. Since only $\frac{1}{3}$ of radiate ($R- G-$) plants in 5F were expected to be *RR*, the choice of seven to ten *RR* plants was unlikely to occur at random (for seven, $\chi^2 = 3{\cdot}38$, $P < 0{\cdot}1$; for eight, $\chi^2 = 6{\cdot}00$, $P < 0{\cdot}025$). Although not highly significant, the result suggests that *r* reduces ray number or is linked to a gene that does.

By contrast, obtaining three *GG* parents was consistent with random expectation (expected value 4, $\chi^2 = 0{\cdot}38$), indicating that ray number is independent of *G/g*.

Three of five progenies from crosses of plants with high ray number in 6U (table 4) were all-radiate and two segregated $\frac{1}{4}$ gibbous plants. Similarly, two of three progenies from crosses of plants with low ray number were all-radiate and one had $\frac{1}{4}$ gibbous plants. This comparison again implies the independence of *G/g* from ray number.

Selection for ray number in Layia glandulosa

Selection experiments were done to test the possibility that polymorphism in *L. glandulosa* might be another source of genetic variation in ray number in hybrids. Two plants from subsp. *glandulosa* with 13 rays on the first head and more than eight rays on subsequent heads were inter-crossed, and a plant with 13 rays was crossed to one with eight rays. Average ray number in the "13 × 13" progeny but not the "13 × 8" progeny exceeded that in a contemporaneous unselected sample from subsp. *glandulosa* (6GG vs. 6GA, table 5; $F = 9{\cdot}54$, $P < 0{\cdot}01$). The four plants with highest ray number, based on the first two heads, were crossed and their progeny again exceeded an unselected sample in ray number (8G vs. pooled 8GA, 8GB, table 5; $F = 17{\cdot}4$, $P < 0{\cdot}001$).

Previous tests have indicated that cold temperature increases ray number (unpublished). Thus the high ray numbers seen in the unselected subsp. *glandulosa* sample of spring 1987 probably resulted from colder growing conditions. This situ-

Table 5 Means and coefficients of variation for number of rays on first head in progenies of plants selected for high and low ray number in *L. glandulosa*

		n	Mean	CV
Selection for high ray number in subsp. glandulosa, *Fall 1986*				
6GA	"13 × 13" both parents high	69	9·1	16
6GB	"13 × 8" one parent high	56	7·9	6
6GG	unselected	27	8·1	11
Selection for high ray number in subsp. glandulosa, *Spring 1987*				
8GA	both parents high	38	12·1	18
8GB	both parents high	38	12·7	11
8G	unselected	31	10·8	16
Selection for low ray number in subsp. lutea, *Spring 1987*				
8LL	both parents low	55	7·6	10
8L	unselected	30	8·8	14

ation prevents determining whether the second selection for high ray number achieved any further advance over the first selection.

Two plants with only five rays per head were selected from subsp. *lutea* and crossed; the progeny had significantly fewer rays than a contemporaneous unselected sample from subsp. *lutea* (8L vs. 8LL, table 5; $F = 33{\cdot}40$, $P < 0{\cdot}001$).

These results confirm that there is genetic variation for ray number within *L. glandulosa*. A similar result may be expected for *L. discoidea*. Thus, some of the variation in ray number in F_1 plants may be due to heterozygosity of one or both parents, and new variation may be introduced with each generation of backcrossing.

Fibonacci numbers

The modal values for number of rays per head on the first four heads of plants grown in spring 1985 were Fibonacci numbers, *i.e.*, members of the series 1, 1, 2, 3, 5, 8, 13, 21, . . . , each number being the sum of the preceding two. Sixty-two per cent of *L. glandulosa* (3G, 3L) heads had eight rays and 42 per cent of F_1 (3A, 3K, 3B) heads had five rays. In the backcross to *L. discoidea* (3F, 3R), the mode for the first four heads was zero, but, for first heads only, the mode was three. The most interesting results were for the backcrosses to *L. glandulosa* (3E, 3P; fig. 4). Progeny 3P had mean 5·5, median 5·0, a pronounced mode at five, a slight mode at eight and was not significantly skewed ($g_1 = -0{\cdot}22$, $g_1/se_g = -1{\cdot}54$). In contrast, progeny 3E, with mean 6·8, had median 7·0, a pronounced mode at eight, a slight mode at five, and highly significant skewness ($g_1 = -0{\cdot}55$, $g_1/se_g = -4{\cdot}30$, $P < 0{\cdot}001$). Thus, the tendency for heads to have a Fibonacci number of rays may impart a bias away from the normal distribution usually expected for polygenic characters.

Number of gibbous florets per head

The genetic similarity of radiate and gibbous plants predicts that the same genes govern ray and gibbous floret numbers. Progenies that segregate both radiate and gibbous plants have both floret types in similar numbers per head (tables 3 and 4), consistent with the prediction.

Discoid plants on Layia glandulosa genetic background

Backcrosses to both subspecies of *L. glandulosa* were initiated to transfer genes *r* and *g* from *L. discoidea*. BC_n plants were all radiate $R-G-$ with five to eight rays per head, sometimes fewer on the first head. Plants with few rays on early heads were selected as parents from the BC_1. Two BC_2 progenies to each subspecies of *L. glandulosa* were grown (figs. 2 and 3). Pairs of plants with few rays from each BC_2 were crossed and seven progenies were grown (figs. 2 and 3). One progeny, 6A, included a substantial number (15/115) of discoid plants. Six progenies produced from the discoid plants in 6A were grown and all except one were uniformly discoid (data not shown).

Corolla size

Rays of F_1 plants were smaller than in *L. glandulosa* and those of backcross plants were smaller yet (Ford and Gottlieb, 1989), showing that *L. discoidea* has genes affecting ray size. Size did not continue to decline with subsequent generations

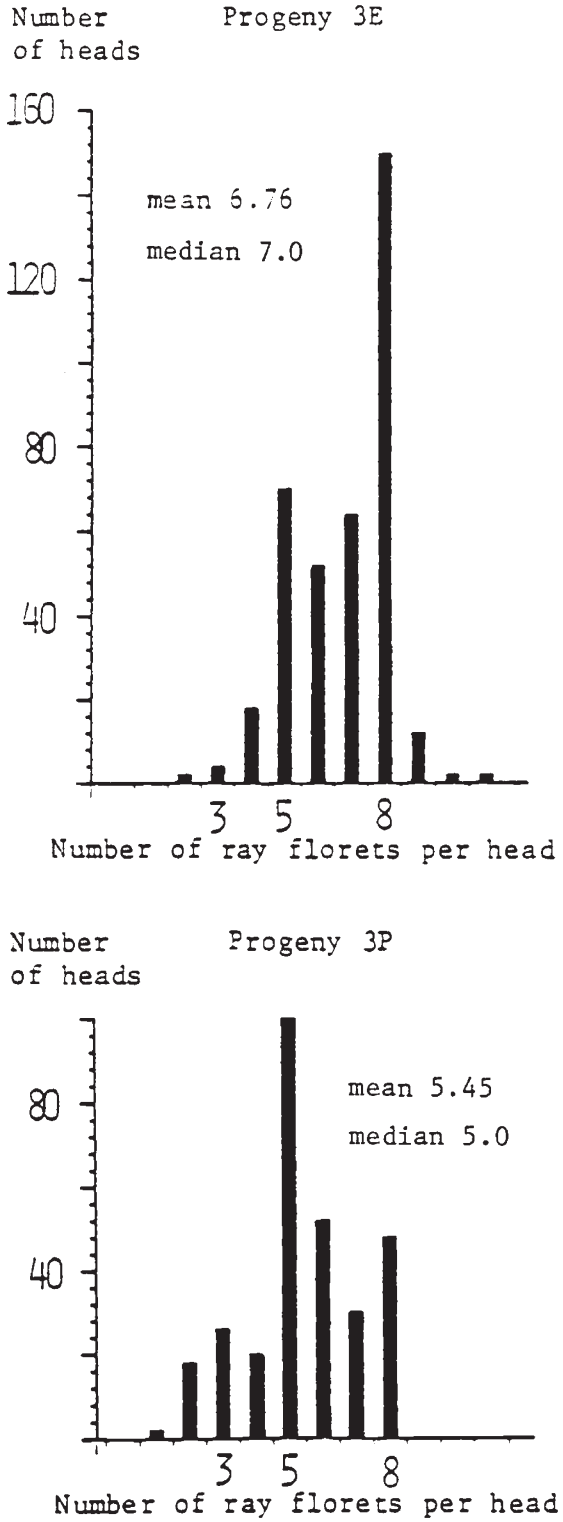

Figure 4 Number of ray florets per head in first generation backcrosses to *L. glandulosa*, counting the first four heads from every plant.

of backcrossing (Ford and Gottlieb, 1989). Ligule shape also varied (fig. 1K). In progeny 6F ligules varied from slightly longer than wide (14 mm × 12 mm) to twice as long as wide (16 mm × 8 mm).

Gibbous corolla size also varied with genetic background. Gibbous plants were first seen in progenies with an 87 per cent *L. discoidea* background. Crosses of such gibbous plants to *L. discoidea* and to subsp. *lutea* produced plants on a 94 per cent *L. discoidea* background with tiny gibbous florets, and plants on 54 per cent *lutea* background with large gibbous florets (fig. 1G).

Corolla shape variants

Disk florets with small sectors of ray-like tissue (chimeras) were present on many F_1 plants, as well as some rays with extra lobes, asymmetric ligules, or tubular corollas. In F_1 progeny 2A grown in fall 1984, ten out of 24 plants had three or more irregular florets on the first four heads, but when the progeny was re-grown in spring 1985 (3A) the incidence of such plants was much lower, two out of 41, suggesting unstable expression of threshold traits. Tubular rays were seen in only a few F_1 plants but two generations of selection yielded larger and more symmetrical tubes (fig. 1I). Thus, the species may be polymorphic for genes which increase the stability and expression of variant florets. Opposite-lobe rays (fig. 1J) also proved to be heritable. True-breeding lines have not yet been produced for either type. Gibbous florets also showed variation in lobe number (four instead of five) and shape.

Clasping involucral bracts

The involucral bracts of hybrid radiate plants generally were folded only partly around the subtended ovary. But, the *RR GG* plants of progeny 6Q and their descendents had bracts fully enclosing the ray achenes, as in *L. glandulosa*. Since this trait had not been selected, it may be an effect of the *RR GG* genotype. Further tests have not yet been made.

In F_2 progenies 5U and 7B, clasping involucral bracts lacking axillary florets were seen. These "empty phyllaries" were heritable and two true-breeding lines have been established.

DISCUSSION

Our experimental hybridization of *L. discoidea* and *L. glandulosa* confirms the conclusion of Clausen,

Keck and Hiesey (1947) that ray presence/absence is governed by two loci, here designated R/r and G/g, that assort independently. We also confirmed their conclusion that *L. discoidea* has genes reducing ray number and size and modifying ray colour in crosses with both the white-rayed subsp. *glandulosa* (noted by Clausen) and the yellow-rayed subsp. *lutea*.

However, our much more extensive crossing programme allowed a number of additional conclusions. Clausen depicted ray presence/absence as a simple, dimorphic character, admitting only certain modifiers that did not affect a strict dichotomy of ray and disk florets. It was this representation which led us initially to hypothesize that discoid heads (capitula) result from the abortion of ray primordia. Instead, our work revealed a wealth of variant, intermediate, or recombinant forms. These include (1) gibbous florets, (2) rays with normal corollas but varying amounts of pubescence, pappus and rudimentary anthers, (3) tubular rays and opposite-lobe rays, (4) chimeras, (5) slightly distorted ray and disk florets, and (6) the irregular intermediate florets seen in many of the "polymorphism test" progenies (table 2). Our analysis indicates that gibbous florets are produced by the homozygous recombinant genotype *RRgg* and, weakly, by *Rrgg*, while rays with ovary pubescence and/or rudimentary stamens occur on $R-Gg$ heterozygotes. Tubular and opposite-lobe rays have been demonstrated to be heritable but their genetic basis has not yet been worked out. The other variant types are unstable in their expression, likely to appear only as one or a few odd florets on a plant. They are presumably threshold traits of low heritability but, since we have seen them only in hybrids, they must have a genetic basis involving some combination of *L. glandulosa* and *L. discoidea* genes.

The discovery that *L. discoidea* is polymorphic for genes affecting ray presence/absence was unexpected and added another dimension of interest to the work, as well as another dimension of difficulty to the attempt to produce homozygous stocks for developmental studies. We refer to the *L. discoidea* allele promoting ray presence as "*G*" in the absence of any definitive evidence to the contrary. But, the test progenies that had more than $\frac{1}{4}$ radiate plants, and thus presumably an *rrGg* or *rrGG L. discoidea* parent, often had many plants with intermediate florets of a type not generally seen in our main backcrossing program (table 2). This suggests the *L. discoidea* allele in question may prove to be some variant *G'*.

The same experiments which confirmed polymorphism in *L. discoidea* also confirmed that at least one other *L. discoidea* gene contributes to ray absence. Several experiments pointed to a close relationship between ray presence/absence and ray number, probably involving effects both of *r* and the additional postulated gene. At the same time, our selection experiments confirmed that there is genetic variation for ray number within *L. glandulosa*. Ray florets, gibbous florets, tubular ray florets and opposite-lobe ray florets are all subject to similar variations in number, size and colour.

Given the many genetic differences between the species, the unexpected invisible polymorphism in *L. discoidea*, and the problems of crossing self-incompatible plants (including inbreeding depression), it is not to be expected that a study of this type will produce such neat results as are obtained with crosses of inbred lines of crop species. For example, the 13:3 radiate versus discoid F_2 segregation described by Clausen and coworkers is not consistent with our results; possible explanations for the discrepancy include the use of different criteria for classifying phenotypes, genetic differences between subsp. *glandulosa* (used for their work) and subsp. *lutea* (used for most of ours), and genetic differences within *L. discoidea*.

The emerging picture of the genetic differences between *L. discoidea* and *L. glandulosa* fits neither of the stereotypic models of major genes or polygenes. Clearly, many genes are involved, but most have qualitatively distinguishable effects. These effects resist facile classification: the genes *r* and *g* which govern "presence/absence" also function as modifiers of number and morphology; one or two other genes governing number are also implicated as having "presence/absence" effects, at least on some backgrounds. A complete range of corolla sizes is seen but rays less than about 4 mm in length are rarely regular in shape (data not shown), suggesting that genes with quantitative effects on dimension also have other effects below some threshold.

Despite these complexities, the strategy of using selection to reduce background genetic variation has allowed us to obtain clear results at least for the two genes R/r and G/g of largest effect. Selection for high ray number allowed us to continue to recover clear segregations of radiate and discoid plants after four generations of backcrossing to *L. discoidea*. The BC_2F_2 background facilitated recognition of the gibbous phenotype and may have been critical to our success in obtaining definitive results from the crosses of gibbous plants to subsp. *lutea* and *L. discoidea*. Similar procedures for identifying additional specific genes are evidently feasible, but more work is necessary

as the effects to be isolated are increasingly small.

Since the evolutionary change under examination is the loss of rays in *L. discoidea*, it is important to note that the complex set of genetic differences observed does not imply that many gene substitutions were *required* to effect that loss. Our success in transferring discoid heads to an 87 per cent *L. glandulosa* background by selecting BC_1 and BC_2 plants with few rays and intercrossing them implies that relatively few *L. discoidea* genes were required. In fact, our model for the effects of R/r and G/g implies that an *RR* to *rr* substitution alone would suffice to confer absence of rays to plants with an *L. glandulosa* genetic background. Additional tests are required before the model can be considered rigorously proven, but it is consistent with all available data.

It is initially surprising that a species that lacks rays has modifiers of ray size, shape, number and colour and polymorphism for genes affecting ray presence/absence. One possible explanation is occasional hybridization with *L. glandulosa*. In 1983 the nearest populations of *L. glandulosa* that we were able to find were 25 miles from *L. discoidea* and differed by a thousand feet in elevation, but nearer sites have been reported (UCB herbarium). Also, small rays occur at low frequency in at least two *L. discoidea* populations. However, the population of *L. discoidea* used for the present experiments was remote from these and in another river valley. We think it more likely that some *L. discoidea* genes modifying ray development in hybrids have other functions in *L. discoidea* and are maintained for that reason. Also, some *L. discoidea* alleles may be inactive, resulting in the loss of functions that are not essential but serve to stabilize or canalize ray development.

Several other reports have claimed single gene control of ray presence/absence in various Compositae: *Senecio vulgaris*, Trow, 1912; *S. squalidus*, Ingram and Taylor, 1982; radiate *Haplopappus aureus* × discoid *H. venetus* subsp. *venetus*, Jackson and Dimas, 1981. In *S. squalidus*, some heterozygotes have tubular rays and "bilabiate" rays (with one or two lobes opposite the three-lobed ligule) and aborted ray stamens (Ingram and Taylor, 1982). Fick (1976) reported that tubular ray florets are conferred by a single recessive gene in *Helianthus annuus*. Many other instances of evolutionary loss of rays are known but have not been subject to genetic study.

The fact that many independent instances of loss of rays have occurred in the Compositae suggests that this change is easy to accomplish. That is, many mutations can modify head development so that rays are not produced, but viability and fertility are not significantly reduced. In this connection, the discovery of gibbous florets is particularly interesting. Phenotypically, they are conspicuously different from rays. Yet, their appearance is uniform and their fertility unimpaired, even though presumably there has never been any selection to canalize this phenotype. Thus, significant changes may sometimes be assimilated into the ontogenetic process without physiologically adverse pleiotropic effects.

One of our objectives in undertaking the crossing program described, was to produce stocks that can be used to learn how, developmentally, changes in head phenotype are constructed. Although formal developmental studies of the hybrid lines have not yet been completed, the genetic results have some developmental implications. For example, the smaller size of *L. discoidea* heads (fig. 1A) suggests the hypothesis that ray absence results from small head size; this was rejected by the transfer of genes for ray absence to the *L. glandulosa* genetic background with resulting formation of large discoid heads.

The widespread occurrence of spiral phyllotaxy in dicotyledonous plants suggests that the mechanisms governing placement and initiation of primordia are independent of their subsequent development. The observation that modal values for ray number are Fibonacci numbers is consistent with the hypothesis (*e.g.*, Bachmann, 1983) that floral development is guided by radial gradients of morphogens on a composite receptacle. The connection lies in the fact that the number of primordia which fit in a band around the receptacle tends to be a Fibonacci number, depending on the relative sizes of primordia, receptacle and band width. A review of Fibonacci numbers in plant development, and a rationale in terms of geometric constraints on close packing of primordia, are provided by Mitchison, 1977. In terms of this model, gene *r* might reduce the inductive band width, resulting in a reduction of ray number in *Rr* heterozygotes and an absence of rays (band width too small) in *rr* homozygotes. For now, the model has only heuristic value, demonstrating a possible mechanism combining quantitative and qualitative effects, and illustrating the importance of developmental analysis to reveal geometric or other physical factors intervening between gene products and the final phenotype. It also suggests new questions, *e.g.*, does *r* reduce the total number of florets or only the number (proportion) of rays? Answering this question would depend on developing lines isogenic for all other genes affecting floret number.

A more specific model for the effects of *r* was suggested by comparison of head development in the two species (Gottlieb and Ford, 1987). In *L. glandulosa*, involucral bract primordia, paired receptacle bract/disk floret primordia and finally disk floret primordia without subtending bracts appear sequentially in helical fashion. The ray primordia appear more or less simultaneously in the axils of the involucral bracts after all the involucral bracts are present. Thus, pairs of disk floret and receptacle bract primordia begin growth together while a ray primordium is delayed behind its associated bract. In *L. discoidea*, there are no bracts with delayed axillary primordia. If the delayed growth of peripheral floret primordia is necessary to permit them to receive special biochemical or biophysical signals, then this change in timing may cause the peripheral florets of *L. discoidea* to develop as disk florets. This model is useful because it generates testable predictions, *e.g.* (1) rays on all genetic backgrounds should exhibit a similar delay in growth, (2) if the delay results from the action of *R* then it should also characterize the peripheral primordia of gibbous plants (*RR gg*). These will be addressed in our developmental study.

Comparison of radiate *RR GG* plants with gibbous *RR gg* plants might suggest that *g* acts as a switch, shifting several components of floret ontogeny as a single unit: corolla shape, ovary pubescence, pappus and anthers. But, in *RR Gg* plants, a single *g* allele suffices to switch some traits (ovary pubescence) but not others (corolla shape). Further, although *g* is implicated in the occurrence of both ovary pubescence and rudimentary stamens in rays, either trait may occur without the other. Ray pappus, when it occurs, is always associated with ovary pubescence, but not vice versa. Thus, it is important not to oversimplify the "switch" concept. The gibbous phenotype is not in any sense specified by *g*, but is an epigenetic consequence of the interaction of *g* with many other genes governing floret development. Developmental studies must include heterozygotes as well as homozygotes, and both on a variety of genetic backgrounds, in order to exploit the power of genetic analysis to separate the various components of floret development. The "empty phyllary" plants may help reveal what connection, if any, exists between the later stages of bract and floret development.

Characterization of evolutionary morphological changes as major or minor is illusory unless founded on genetic analysis: the demonstration that the absence of ray florets in *L. discoidea* is conferred by a simple genetic difference shows that this was not a large change despite the accretion of a considerable number of differences between the species. The discovery of gibbous florets has particular interest because it demonstrates that novel combinations of developmental processes can be readily assimilated without evident adverse effects. The complex admixture of genes with large and small, qualitative and quantitative, effects may prove typical as more instances of morphological evolution are subjected to intensive genetic analysis.

REFERENCES

BACHMANN, K. 1983. Evolutionary genetics and the genetic control of morphogenesis in flowering plants. *Evolutionary Biology, 16*, 157–208.

CLAUSEN, J., KECK, D. D. AND HIESEY, W. M. 1947. Heredity of geographically and ecologically isolated races. *Am. Nat., 81*, 114–133.

FICK, G. N. 1967. Genetics of floral color and morphology in sunflowers. *J. Hered., 67*, 227–230.

FORD, V. S. AND GOTTLIEB, L. D. Morphological evolution in *Layia* (Compositae): Character recombination in hybrids between *L. discoidea* and *L. glandulosa*. *Syst. Bot., 14*, 284–296.

GOTTLIEB, L. D. 1984. Genetics and morphological evolution in plants. *Am. Nat., 123*, 681–709.

GOTTLIEB, L. D. AND 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*, Cambridge University Press, Cambridge.

GOTTLIEB, L. D., WARWICK, S. I. AND FORD, V. S. 1985. Morphological and electrophoretic divergence between *Layia discoidea* and *L. glandulosa*. *Syst. Bot., 10*, 484–495.

INGRAM, R. AND TAYLOR, L. 1982. The genetic control of a non-radiate condition in *Senecio squalidus* L. and some observations on the role of ray florets in the Compositae. *New Phytol., 91*, 749–756.

JACKSON, R. C., AND DIMAS, C. T. 1981. Experimental evidence for systematic placement of the *Haplopappus phyllocephalus* complex (Compositae). *Syst. Bot., 6*, 8–14.

MITCHISON, G. J. 1977. Phyllotaxis and the Fibonacci series. *Science, 196*, 270–275.

TROW, A. H. 1912. On the inheritance of certain characters in the common groundsel—*Senecio vulgaris* L.—and its segregates. *J. Genet., 2*, 239–276.