YELLOW POLLEN—A NEW GENE IN *PISUM*

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1. INTRODUCTION

PEA pollen is normally orange in colour. However, a plant having pollen of a light yellow colour was noticed amongst seedlings raised from some old stocks of the variety Vinco. The yellow colour bred true in subsequent generations. Crossing with an orange variety indicated a monofactorial difference with complete dominance of orange. Additional crosses were then undertaken to confirm this preliminary finding and establish if possible the linkage relations of the new locus.

2. MATERIALS AND METHODS

Details of the pure varieties used in the investigation are given in table 1. Lines, 2, 6, 21, 25, 31 and 34 were kindly supplied by Professor Robert Lamm, Agricultural College of Sweden, Alnarp. The source of the original Vinco seed in this collection is unknown. Details of the genes used as markers may be found in a review of pea genetics by Yarnell (1962). The marker genes are not grouped according to the characteristic controlled but follow the gene sequence in the linkage map drawn up by Lamprecht (1961). Line 51*y* was used as the male parent in crosses with Lines 2, 6, 21, 25, 31 and 34. The cross numbers corresponding to these six crosses are respectively 64, 63, 65, 66, 67 and 70. F_1 plants were raised in the field in order to obtain large progenies. F_2 plants were grown in the glasshouse in boxes or tins of a vermiculite—dolerite mixture. Under these conditions germination and survival are excellent but progenies small. Over 98 per cent. of all F_2 seeds planted grew to a stage where pollen colour could be recorded. The results have been analysed by chi-squared following the methods of Mather (1963). Recombination values have been estimated by the product-ratio method using Immer's (1930) tables.

3. RESULTS

(i) *Segregation for pollen colour*

Although some variation is observed in the shade of both orange and yellow pollen the difference between the two is usually such that a plant may be classified at a glance. Segregation for pollen colour is given in table 2. The pollen colour of F_1 plants was similar to that of the orange parent. Segregation in each of the six crosses is an acceptable monohybrid ratio. The heterogeneity between crosses is not significant and the combined data show beyond doubt the monofactorial nature of the pollen colour difference. It is proposed to denote the recessive allele for yellow pollen by the letters *yp*.

(ii) *Joint segregation*

The joint segregation for pollen colour and various markers is set out in table 3. Where one marker has been recorded in two or more crosses, the combined figures only are given together with the chi-squared for heterogeneity between crosses. The genes *P* and *V* must both be present for the formation of a strong membrane inside the pod. The genotypes *Pv*, *pV*

TABLE 1

Details of the pure lines used in the investigation

Line	Genotype for the markers used															Other names
	a	*i*	*wa*	*s*	*wb*	*k*	*m*	*st*	*b*	*le*	*v*	*cri*	*wlo*	*p*	*pl*	*r*	
2	+	+	+	+	+	+	*m*	+	+	*le*	*v*	+	+	*p*	*pl*	+	Graue niedrige*
6	*a*	*i*	*wa*	+	+	+	*m*	+	+	*le*	+	+	+	+	*pl*	+	de Haan 204·1†
21	*a*	+	+	+	+	+	*m*	+	+	*le*	+	*cri*	+	+	*pl*	+	Lamm L21
25	*a*	+	+	*s*	*wb*	+	*m*	+	*b*	*le*	+	+	+	+	*pl*	+	Lamm L25
31	+	+	+	*s*	*wb*	*k*	*m*	*st*	*b*	*le*	+	+	*wlo*	+	*pl*	+	Lamm L31
34	+	+	+	+	+	+	*m*	+	+	+	+	*cri*	+	+	+	+	Lamm L101
51*y*	+	+	+	+	+	+	+	+	+	*le*	+	+	+	+	*pl*	+	Vinco‡

* Rasmusson Gd, Lamm Line 2. † Lamm Line 6. ‡ Yellow pollen variant.

and *pv*, which lack the complete membrane, were not readily distinguishable phenotypically in this instance, and have been grouped to give a 9:7 ratio. With the exception of *b* all markers have segregated in agreement with expectation. The deviation for *b* is just significant at the 5 per cent. level but there is no reason to suspect this is anything more than chance. The *yp* segregations are all satisfactory but the heterogeneity chi-squared between crosses is just significant in two cases. This arises from Cross 65 which has a slight, though acceptable, excess of plants with yellow pollen. Considered overall, as shown in table 2, there is no serious evidence of between cross heterogeneity for the segregation of pollen colour.

TABLE 2

Segregation for pollen colour in F_2

Cross number	Orange	Yellow	Total	Number of seeds planted	χ^2_1 Segregation
63	182	47	229	230	2·45
64	129	48	177	192	0·42
65	159	69	228	230	3·37
66	149	42	191	193	0·92
67	444	127	571	578	2·32
70	146	42	188	192	1·39
Combined	1209	375	1584	1615	1·48

χ^2_5 for heterogeneity between crosses 9·39.

The chi-squareds for the joint segregation of *yp* with the group III markers *st* and *b* are both highly significant. The recombination value for the pair *st-yp* based on the data in table 3 is 27·62% ± 3·82%. As there are no double recessives of the type *b yp* and the numbers for the joint segregation are in agreement ($\chi^2_2 = 5{\cdot}3$) with the 2:1:1 ratio expected in a cross in repulsion involving two factors which are absolutely linked, the distance *b-yp* is apparently zero. However, such a conclusion is quite unjustified with these small numbers as crosses in repulsion are highly inefficient for obtaining recombination values for closely linked genes (Mather, *loc. cit.*). With the same numbers in the other classes even one plant in the class *b yp* would give a recombination value of 7·4 per cent. On the basis of no double recessives in 713 plants, we can be 95 per cent. confident that *yp* is within 12·95 units of *b*. The chi-squared for the joint segregation of *yp* with the other group III marker *m* is very small indicating free recombination and this is reflected in the recombination value for *m-yp* of 49·26% ± 3·11%.

Joint segregations of the markers *b*, *st* and *m* are given in table 4. Lamprecht (1946) has carried out an extensive investigation of the linkage relationships of the group III genes and the present results are consistent with his findings.

These results show clearly that *yp* is in linkage group III in the vicinity of *b*. However they do not permit either the determination of the relative position of the *yp* locus with respect to *b* and *st* or the recombination value for *b-yp*.

TABLE 3

Joint segregation in F_2 for pollen colour and various marker genes

Cross Numbers	Phase†	Phenotypic classes				Total	Number of seeds planted	Marker and linkage group		Chi-squared $df = 1$			Heterogeneity chi-squared$_1$ between crosses		
		$+Yp$	$+yp$	$-Yp$	$-yp$					Segregation of marker	Segregation of *yp*	Joint Segregation	Marker	*yp*	Joint Segregation
63, 65, 66	R	372	114	118	44	648	653	*a*	I	0·00	0·13	0·89	0·14	6·61*	1·36
63	R	136	35	46	12	229	230	*i*	I	0·01	2·45	0·00	...	...	...
63	R	141	32	41	15	229	230	*wa*	II	0·04	2·45	1·58	...	...	...
66, 67	R	213	67	55	24	359	457	*s*	II	1·72	0·02	1·23	0·05	1·58	2·18
66, 67	R	467	126	126	43	762	771	*wb*	II	3·24	3·24	1·40	0·06	0·00	0·37
67	R	338	100	106	27	571	578	*k*	II	0·89	2·32	0·27	...	...	...
64, 67, 70	R	330	117	107	36	590	648	*m*	III	0·18	0·27	0·06	1·66	1·50	0·06
67	R	304	117	140	10	571	578	*st*	III	0·49	2·32	27·66***	...	...	...
66, 67	R	352	159	202	0	713	722	*b*	III	4·22*	2·77	80·89***	0·83	0·01	0·08
70	C	113	38	33	4	188	192	*le*	IV	2·84	0·71	2·42	...	...	...
64	R	78	33	51	15	177	192	*v*, *p*	IV, VI	3·00	0·42	1·21	...	...	...
65, 70	R	233	77	74	32	416	422	*cri*	V	0·05	0·32	1·24	0·06	4·44*	0·09
67	R	319	94	125	33	571	578	*wlo*	VI	2·17	2·32	0·33	...	...	...
70	C	107	33	34	8	182	192	*pl*	VI	0·36	0·59	0·89	...	...	...
65, 66	R	237	79	71	32	419	423	*r*	VII	0·04	0·50	1·49	0·80	3·79	2·43

† Coupling C, Repulsion R. * $P<0·05$. *** $P<0·001$.

4. DISCUSSION

The F_2 in coupling phase and the backcross are both much more efficient than the F_2 in repulsion phase for determining the recombination value of two closely linked genes. The variety Vinco in which the mutant *yp* first appeared carries the dominants *B* and *St*. The initial crosses were of necessity in repulsion and until a recombinant of the type *b yp* is available the preferable cross in coupling cannot be made. The progenies from the F_2 plants are far too small to give a reasonable chance of genotyping the F_2. However, the chance of obtaining the double recessive *b yp* is greatly

TABLE 4

Joint segregation of the markers b, st *and* m

Cross	Phase	Phenotypic classes				Total	Planted	χ^2_1			Recombination value
		BSt	*Bst*	*bSt*	*bst*			Seg. *b*	Seg. *st*	Joint	
67	C	341	64	80	86	571	578	5·1*	0·5	78·8***	27·8% ± 2·2%
		StM	*Stm*	*stM*	*stm*			Seg. *st*	Seg. *m*		
67	C	140	39	39	15	233	264	0·4	0·4	0·8	45·5% ± 4·6%
		BM	*Bm*	*bM*	*bm*			Seg. *b*	Seg. *m*		
67	C	129	42	50	12	233	264	0·3	0·4	0·7	54·3% ± 5·1%

* $P<0.05$. *** $P<0.001$.

increased in progenies from the single dominant classes in the F_2. The work will be continued by growing these F_3 plants and further F_2 plants from Cross 67.

Yellow pollen should prove of some use as a marker as the colour difference is readily distinguishable and can be used in more varied circumstances than the adjacent gene *b*, which cannot be detected in the absence of *A*, the basic factor for anthocyanin production.

5. SUMMARY

1. Crosses between a yellow pollen variant of the variety Vinco and several lines having normal orange pollen have shown a single factor difference with complete dominance of orange.

2. The recessive allele for yellow has been given the symbol *yp*. The *yp* locus is in linkage group III in the vicinity of *b* but the order of the loci *b*, *yp* and *st* and the distance *b-yp* cannot be determined from the present data.

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6. REFERENCES

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TWITTERING—A VOICE MUTATION IN *MICROTUS*

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IT has been impressively demonstrated that behaviour, like structure, is genetically determined. It must, therefore, be affected by mutation. The isolation (" Kaspar-Hauser ") experiments of ornithologists in particular have demonstrated that a great number of vocal performances in animals are genetically determined, but little is known about mutations affecting them. As rare mutations are most often observed in large laboratory stocks, rodents may be suitable subjects, provided that the breeder pays attention to the vocalisations of his animals (which is seldom the case).

So-called " singing " in mice (*Mus musculus*) has been observed on several occasions and was studied by Dice (1932). " The musical mouse produced a song which was bird-like in quality, but weak in volume. . . . Generally the song consisted of a series of pleasing musical chirps and twitters. . . . Some of the F_1 offspring had a faint song, which might better be called a ' chitter '. The chitter may be described as a faint rapid trill, quite musical in character." This was quoted by Grüneberg (1952), who himself continues: " Neither in the F_2, nor in the F_3 nor in repeated backcrosses of daughters, grand-daughters, etc. to the original male were animals produced whose vocal performances equalled that of the original male. Thus, while its singing habit was not inherited, the weak ' chitter ' in its descendants was probably determined genetically, though the mode of its inheritance could not be ascertained."

The present author has discovered a similar phenomenon in the Continental European Vole. The effect is genetically determined and appears to be the first well-established demonstration of a mutation directly affecting a behavioural character.

1. ORIGIN AND DESCRIPTION

A study of the mode of inheritance of several coat colour mutations found in nature has been carried out on the author's laboratory population of voles for several years (Frank and Zimmermann, 1957). These included recessive (*s*) and dominant (*W*) spotting. After inbred lines had been established following crossbreeding individuals with these two mutations, two females (related cousins) with deviant vocalisation appeared among the descendants in 1957.

Whereas the normal threatening call of *Microtus arvalis* is a monosyllabic, loud and shrill squeak (something like " dyeep "), the deviant animals