NOTES AND COMMENTS

ABERRANT SEGREGATION OF A GENE IN THE RASPBERRY AND ITS ASSOCIATION WITH EFFECTS ON SEED DEVELOPMENT

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1. INTRODUCTION

ABERRANT segregation of the marker genes *H*, *T* and *S* in the raspberry has been attributed to linkage with three semi-lethal genes designated *Wh*, *Wt* and *Ws* respectively (Jennings, 1967). Gene *S* determines the presence or absence of cotyledonary glands, so its segregation can be studied in large progenies at the earliest possible stage after germination. The results of such a study suggested that in some crosses aberrant segregation occurred because either gene *Ws* or gene *S* itself affected seed dormancy through effects on endosperm development, while in others it occurred through effects on competition between pollen tubes of alternative genotype. This paper assesses the evidence for this hypothesis and discusses its implications.

2. MATERIALS AND METHODS

The parents of the progenies studied include the cultivar Burnetholm, No. 3B45 from the cross Burnetholm $\times$ Malling Jewel, Nos. S29/11, S29/91 and S29/97 from the self of 3B45, AR1 from the self of Burnetholm and 511/12 from the cross Lloyd George $\times$ AR1. Burnetholm, 3B45, S29/11, S29/91 and 511/12 are heterozygous for gene *S*, and S29/97 and AR1 are homozygous recessive. There were four series of crosses which were as follows:

1. A 5×5 diallel made in 1964 and known as diallel 1, involving the parents Burnetholm, 3B45, 511/12, S29/97 and AR1. Crosses were made in the field and auxin (50 p.p.m. of equal parts betanaphthoxyacetic acid and parachlorophenoxyacetic acid) was applied 7 days after pollination to alternate fruits of the fruiting laterals. The seed was sown in pots in November 1965 and kept in an unheated glasshouse. Seedling emergence commenced in March 1965 and segregation was recorded until emergence ceased in May. Twenty-one of the 25 possible parent combinations gave families segregating for gene *S* and a total of 13,809 seedling were classified.

2. Material from a similar diallel made in 1965 and known as diallel 2. It was handled in exactly the same way except that the crosses were made in an unheated glasshouse. Only three of the parents (Burnetholm, 511/12 and AR1) gave progenies segregating gene *S* and 4037 seedlings were classified from six of the nine segregating families.

3. Six crosses made in 1963 in which Burnetholm and 3B45 were the seed parents and S29/11, S29/91 and S29/97 the pollen parents, giving a total of 3093 seedlings.

4. Three experiments to study the effects of pollen dilution, two of which have already been described (Jennings and Topham, 1971). Pollen was diluted to 50 per cent. or 75 per cent. by weight with talc and pollinations were then done as uniformly as possible, applying similar quantities of mixture or undiluted pollen to the stigmas.

For the diallels, variations in pyrene set, seed structure and seedling emergence have already been described, together with the methods used for measuring them (Jennings, 1971*a, b, c*; Jennings and Topham, 1971). The data from the diallels were analysed by fitting constants to quantitative data on the percentage of recessives obtained. The model used was:

$$\% \text{ recessive } s = u + f + m + a + fm + fa + ma,$$

where u is the mean, f , m and a are respectively the main effects of the female and male parents and the main effect of auxin, and the other effects are first-order interactions between these factors. The data were transformed to logits to base e and the constants fitted by the method of maximum likelihood, using an iterative procedure. By this method each constant has its own standard error obtained by the variance/covariance matrix.

3. RESULTS

Segregation

The results for segregation of gene S in diallels 1 and 2 are given in table 1 and analyses of the factors determining it are given in table 2. These show that the largest deviations from expected segregation ratios were associated with paternal effects, use of Burnetholm pollen giving a deficiency of recessives in both diallels and use of 3B45 and 511/12 pollen giving excesses in diallel 1. In diallel 1 there were no significant paternal effects for the two pollen parents homozygous for gene s , but in diallel 2 pollination with AR1 (ss) gave significant deficiencies of recessives. There was a deficiency of recessives due to the maternal effect of AR1 in diallel 1, while the deficiency due to the maternal effect of 511/12 in both diallels was small but significant. Eight of the maternal $\times$ paternal interactions were also significant in diallel 1, but none were significant in diallel 2.

The main effect of auxin on segregation was not significant, but there were significant interactions between both maternal and paternal effects and auxin, even though the auxin was not applied until 7 days after pollination.

Seed dormancy

In diallel 1, S phenotypes tended to emerge later than s phenotypes (table 1), giving an average delay of 6.14 per cent. Indeed, late emerging S seedlings so often reduced the departure from the expected ratio that more good fits may have been obtained if emergence had proceeded beyond the low average value of 36 per cent. Possibly heterozygotes tended to emerge earlier than homozygous dominants, because the emergence time of the dominant forms was on average 8.22 per cent. later than that of the recessives in the nine families which segregated both homozygous and heterozygous dominants, and only 5.25 per cent. longer in the twelve which segregated only heterozygous dominants. This difference was not significant, however. A different result was obtained where Burnetholm was the pollen parent;

TABLE 1

Segregation of S and s forms and the mean number of days required for their emergence in diallels 1 and 2 (emergence time calculated from the date when emergence commenced)

	Segregation				Emergence Time	
	No. seedlings*		% Recessives		S	s
	C	T	C	T	(Combined C + T)	
<i>Diallel 1</i>						
B'hm × B'hm	323	430	8.05	4.88	28.96	28.25
B'hm × 3B45	200	188	26.00	34.57	33.71	31.08
B'hm × 511/12	234	241	21.37	28.22	34.63	32.94
B'hm × S29/97	355	366	46.48	50.27	26.54	23.68
B'hm × AR1	387	417	51.68	47.00	36.70	32.79
3B45 × B'hm	288	306	4.51	5.22	35.45	35.43
3B45 × 3B45	257	276	29.57	24.64	27.78	22.79
3B45 × 511/12	217	281	40.09	26.69	31.26	28.36
3B45 × S29/97	235	241	52.77	47.30	26.15	25.89
3B45 × AR1	139	188	43.17	46.28	34.81	31.83
511/12 × B'hm	217	264	2.30	5.30	44.58	36.54
511/12 × 3B45	250	223	24.40	27.80	41.63	37.80
511/12 × 511/12	180	201	27.22	28.36	46.88	47.01
511/12 × S29/97	339	454	51.33	49.12	41.00	38.96
511/12 × AR1	276	251	42.39	49.40	42.83	40.28
S29/97 × B'hm	272	252	9.93	18.65	30.06	31.33
S29/97 × 3B45	230	365	56.09	52.60	33.90	31.10
S29/97 × 511/12	262	368	50.38	47.55	28.76	25.20
AR1 × B'hm	151	195	9.93	5.13	40.59	36.66
AR1 × 3B45	259	315	56.37	49.52	36.93	37.23
AR1 × 511/12	286	230	50.35	53.91	35.24	37.94
Mean					35.16	33.00
<i>Diallel 2</i>						
B'hm × B'hm	46	34	8.70	8.82	35.47	43.44
B'hm × 511/12	280	240	21.43	25.00	26.94	28.47
B'hm × AR1	466	521	42.70	43.57	19.50	20.19
511/12 × B'hm	395	453	5.82	3.53	21.73	17.58
511/12 × 511/12	356	300	26.40	20.33	28.01	28.70
511/12 × AR1	494	452	43.93	38.50	28.72	28.64
Mean					26.73	27.84

* C and T are from seeds obtained from control and auxin-treated fruits respectively.

recessive forms were deficient in these families, even when they tended to emerge earlier. Prolonged dormancy was associated with large endosperm size in this diallel (Jennings, 1971*c*), so except for these Burnetholm families the results would be explained if the *S* forms had larger endosperms.

In diallel 2, the six families whose segregation was recorded did not show a significant difference in the emergence time of the two forms. However, in this diallel there was no evidence that seedling emergence was limited by dormancy factors attributable to the endosperm (Jennings, 1971*c*).

No effects of the pollen parents on endosperm size were detected in diallel 1, but there was evidence of such effects in six closely related families obtained by crossing Burnetholm and 3B45 with the three sibling plants S29/11, S29/91 and S29/97. In these families dominant forms were in excess among the early emerging seedlings but recessives were in excess by

TABLE 2

Main effects of the parents and their interactions on the percentage of recessive genotypes obtained in diallels 1 and 2. Non-bracketed data are logit transformations. Bracketed data indicate the theoretical percentages predicted if all other effects were zero (u + f or u + m)

Parent	Main effects			
	Diallel 1		Diallel 2	
	Maternal	Paternal	Maternal	Paternal
Burnetholm (<i>Ss</i>)	-0.104 (25.0)	-0.914 (6.2**)	+0.060 (26.0)	-0.822 (5.8**)
3B45 (<i>Ss</i>)	-0.123 (24.3)	0.131 (34.8**)	—	—
511/12 (<i>Ss</i>)	-0.172 (22.5*)	0.134 (34.9**)	-0.039 (22.7**)	-0.021 (23.3)
S29/97 (<i>ss</i>)	0.405 (48.0)	0.506 (47.0)	—	—
AR1 (<i>ss</i>)	0.259 (40.8**)	0.474 (48.6)	—	+0.408 (41.8**)

Interactions between maternal and paternal effects					
Diallel 1					
Maternal parent	Paternal parent				
	B'holm	3B45	511/12	S29/97	AR1
Burnetholm	+0.110*	-0.010	-0.122**	-0.014	+0.038
3B45	-0.033	-0.058	+0.078	+0.040	-0.021
511/12	-0.111	-0.027	+0.020	+0.118**	+0.050
S29/97	+0.116**	-0.012	-0.135**	—	—
AR1	-0.120	+0.185**	+0.262**	—	—

Diallel 2					
Burnetholm	+0.155	—	-0.058	—	-0.039
511/12	-0.100	—	+0.037	—	+0.025

Interactions between auxin and the maternal and paternal effects

	Diallel 1		Diallel 2	
	Mat. × Auxin	Pat. × Auxin	Mat. × Auxin	Pat. × Auxin
Burnetholm	+0.025	+0.059*	+0.056*	-0.038
3B45	-0.038*	-0.003	—	—
511/12	+0.051*	-0.014	-0.036*	+0.018
S29/97	-0.005	-0.048*	—	—
AR1	-0.078**	-0.013	—	+0.008

* and ** indicate significant effects with probabilities of 0.05 and 0.01 respectively.

the time emergence ended, with the largest deviations in the families related to S29/11 and the smallest in those related to S29/97 (Fig. 1). Data from seed dissections (table 3) show that for 3B45 seeds the endosperm was progressively smaller following pollination by S29/11, S29/91 and S29/97, and that a parallel result for the Burnetholm series is precluded only by the high value of the cross with S29/97. However, since endosperm size is estimated by subtracting embryo size from seed size it may have been overestimated in this cross where embryo size was smaller than usual for the seed parent. If this were the case the dissimilarity would be removed and both series would show the strongest selection against gene *S* where the pollen induced the largest endosperm size. It was suggested above that the gene itself promotes the development of large endosperms, so the result suggests stronger selection against the gene when other genetic factors are also acting to achieve this.

Pollen dilution

Further information on the pollen's role was sought in experiments where pollen was diluted with talc. In these experiments neither undiluted nor

FIG. 1.—Accumulative changes in the percentage of recessive genotypes in the six 1963 crosses. The expected percentages are indicated by broken lines.

TABLE 3

Mean embryo and endosperm sizes (mm.²) in six families where gene S affected the germination time

Embryo Size (S.E. difference between two treatment means = 0.0496)

Maternal parent	Paternal parent			
	S29/11	S29/91	S29/97	Mean
Burnetholm	1.562	1.561	1.411	1.511
3B45	1.324	1.497	1.464	1.428
Mean	1.433	1.529	1.437	1.470

Endosperm Size (S.E. difference between two treatment means = 0.0539)

Maternal parent	Paternal parent			
	S29/11	S29/91	S29/97	Mean
Burnetholm	0.905	0.849	0.958	0.904
3B45	1.256	1.178	1.067	1.167
Mean	1.081	1.014	1.013	1.036

diluted pollen of 511/12 induced significant deviations from the expected ratios, but Burnetholm pollen gave large deficiencies of recessives which were progressively reduced by dilution (table 4). This results suggests that *S* pollen tubes derived from Burnetholm were more competitive in the style than *s* pollen tubes, which were consequently more successful when competition was reduced by pollen dilution. Pollen dilution had considerable effects on seed development in the 1968 crosses of this series (Jennings and Topham, 1971), but neither pollen dilution nor gene *S* affected the time of seedling

TABLE 4
*The effect of diluting Burnetholm pollen with talc on segregation of gene *s**

Cross	Year germinated	Undiluted pollen		75% pollen		50% pollen	
		No. seedlings	% <i>s</i>	No. seedlings	% <i>s</i>	No. seedlings	% <i>s</i>
Burnetholm self	1966	80	8.75	—	—	143	16.08
511/12 × Burnetholm	1966	848	4.59	—	—	355	17.46
Burnetholm self	1967	940	3.99	940	10.32	—	—
Burnetholm self	1968	692	3.57	554	9.16	544	15.77
511/12 × Burnetholm	1968	665	6.47	492	6.10	438	14.75
AR1 × Burnetholm	1968	421	10.26	701	15.96	391	32.11

emergence. Pollen dilution improved the total number of seeds emerging by 15 to 20 per cent. but the difference was not statistically significant.

4. DISCUSSION

Except where Burnetholm was the pollen parent, most of the evidence shows that aberrant segregation of gene *S* was due to post-fertilisation effects which had different consequences for the viability and emergence of the alternative genotypes. Different factors affected fruit development and seedling emergence in the two diallel crosses, interactions between the parental effects having considerable influence in diallel 1 but not in diallel 2 (Jennings, 1971*a, b, c*). The difference between the diallels in the relative importance of these interactions for determining the segregation of gene *S* was probably a consequence of this.

A clue to the problem of how the pollen influenced segregation through effects on post-fertilisation development is possibly provided by the evidence of its effect on endosperm size. Histological work on this material showed that variation in early seed development depended upon maternal factors interacting with the pollen, and that slow early development was associated with the attainment of large seed size (Jennings and Topham, unpublished). Since much of the present evidence indicates that *S* phenotypes had relatively large endosperms, it would appear that either *S* pollen had less power to stimulate early development than *s* pollen, or that *S* embryo sacs were less responsive to this early stimulation. Whatever the reason, if *S* forms were weaker in their early development they would tend to set less seed, to develop larger endosperms and endocarps and consequently to emerge later or not at all. Auxin was found to reduce the maternal and maternal interaction effect on seed development, which might explain why it interacted with some of the paternal effects to reduce the deficiencies of *S* forms. This would also explain why auxin influenced the maternal effects of AR1 and 511/12 on segregation, though the fact that it had opposite effects on these two seed parents also needs explaining. There is evidence that the endosperms of AR1 and 511/12 differ in growth substance concentration, which must be optimal for good fruit development (Jennings, 1971*b*), so the different responses may be a consequence of this, especially if gene *S* is in some way involved in determining this difference.

A different explanation is required for Burnetholm pollen. If there were no other differences, the fact that the dominant rather than the recessive

form was favoured could be explained by a difference in linkage between gene *S* and a lethal. But Burnetholm was also different in providing evidence of effects due to pollen competition, and in failing to provide clear evidence of post-fertilisation effects or of any other effects attributable to maternal factors. Possible explanations are that the two alleles of a linked semi-lethal present in Burnetholm differ more strongly in their effects on the pollen's competitive ability than those present in the other parents studied, that the linked gene postulated to affect pollen-tube growth in Burnetholm is not the same as the one postulated for the other parents, or that there are unique interactions between genes governing pollen effects in this variety.

The results for the six 1963 crosses with Burnetholm and 3B45 can be explained by postulating that some of the dominant homozygotes (*SS*) failed to emerge, possibly because gene *S* increased their endosperm size, and that the consequences of this were smaller for the seed parent 3B45, which had a larger average endosperm size than Burnetholm. Heterozygotes must have emerged earlier than recessives to give the results with pollen of S29/97 (*ss*), and this is a likely explanation for the excess of dominants among the early seedlings to emerge in the other families. Heterozygotes were also in excess of recessives in the progenies of S29/97 and AR1, the homozygous recessive parents used in diallel 1. In this diallel, seedling emergence was low and probably limited predominantly to heterozygous and recessive forms, with homozygous dominants most frequently deficient. Indeed, segregation in most of the families studied, particularly in the six 1963 families, would be explained if the optimum level of activity for gene *S* or one linked to it was somewhere between that induced by the heterozygote and that induced by the homozygous recessive, so that the order of germination was either *Ss*→*ss*→*SS* or *Ss*→*ss*, with the extent of the deficiencies occurring in the last class or classes depending on the time when emergence ceased.

A gene allelic to gene *S* occurs in tetraploid blackberries and also affects the time of germination (Haskell, 1955). The stage at which first-year germination ceases could therefore explain variation in the ratios obtained (Jennings *et al.*, 1967): for example, a situation with heterozygotes at an advantage but with recessives slightly superior to dominants would tend to give a germination sequence of *SSss*→(*Ssss*+*SSSs*)→*ssss*→*SSSS*. Deficiency of recessives would then occur if low emergence percentages tended to exclude the last two classes and deficiency of dominants would occur if only one class was affected.

It is interesting to consider the kind of control operating for gene *S* from an evolutionary point of view. Keep (1968) has found that wild raspberries are self-incompatible, but that self-fertile forms have been selected because monocultures of single genotypes are required for domestication. The factors affecting pollen function in Burnetholm could therefore be relics of an incompatibility system, or they could illustrate the kind of resistance to inbreeding commonly found when a self-incompatible species becomes self-compatible during domestication (Rowlands, 1964).

5. SUMMARY

1. Causes of aberrant segregation of gene *S* in the raspberry were investigated in several series of crosses, in two of which auxin was applied to alternate fruits 7 days after pollination.

2. Pollen parents usually had more influence on segregation than seed parents, but the aberrant segregation was probably due to post-fertilisation effects.

3. There was a difference in the seedling emergence time of the alternative phenotypes and failure to emerge probably caused aberrant segregation in some crosses. Variation in seed dormancy was correlated with variation in endosperm size and there was evidence that gene *S* affected the latter.

4. Aberrant ratios associated with the use of Burnetholm as pollen parent were due to other causes. They were improved by dilution of the pollen which probably reduced competition between pollen tubes of alternative genotype.

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