FERTILITY STUDIES IN THE BROAD BEAN (*VICIA FABA* L.)

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Received 27.xi.63

1. INTRODUCTION

PREVIOUS work on the breeding system and fertility studies in *Vicia faba* have concentrated on the agricultural types, in particular the medium to small seeded types commonly grown in Europe (Rowlands, 1958, 1960, 1961; Holden and Bond, 1960; Drayner, 1959). Darlington (1956) has argued that the large flat-seeded horticultural types diverged from the main evolutionary line at least 1500 years ago, and continuous selection for seed size has kept the two main types separate ever since. Pod and seed setting were examined in the horticultural types of bean and were compared with previously published work on agricultural types. In addition, embryological studies were made in an attempt to determine the exact time of zygote abortion following self-pollination (Rowlands, 1960).

2. MATERIALS AND METHODS

Preliminary investigations were made on a large collection of horticultural varieties which included Seville, Windsor and Triple White types. In addition a large, flat-seeded agricultural type, occasionally used fresh for human consumption (Mazagan), was used. No obvious differences in pod and seed setting were observed between any of the varieties and most of the work reported here was done on the two cultivars Triple White (Ad 15) and Mazagan (Ad 134) and their derivatives. The 3Aj progenies were derived from crossing Triple White with a white flowered agricultural type (Ch 170/12/10/1D) (Rowlands 1961) and were based on inbred F₃ plants. Ad 15/CF was an F₃ progeny, again obtained by selfing, from a hybrid between Triple White and a dwarf Seville type (The Sutton). All plants were grown in John Innes potting compost No. 2 in an insect proof greenhouse.

Material for embryological studies was fixed in Randolph's modification of Navashin Fluid—CRAF (Johansen 1940), paraffin sections (L.S.) of ovaries were cut at 15 μ and stained with crystal violet and picric acid (Smith 1934).

3. POD AND SEED SETTING ON SELF-POLLINATION

In the field bean (the agricultural type), it was shown that self-fertility was extremely low, and that selection for improved self-fertility was relatively unsuccessful (Rowlands, 1958, 1960, 1961). Pods and seeds set per plant on selfing 5 lines of broad beans are given in table 1.

3Aj 20/8 and 3Aj 17/10 were two F₃ selfed progenies from a cross between two unrelated types. The difference in mean pod and seed set between the two progenies can be accounted for by the different proportions of sterile plants. Diallel cross analysis of self-fertility in field beans suggested that the necessity for tripping of the flower was

determined by recessive alleles whereas the ability to set seed without tripping behaved as a dominant character (Rowlands, 1960). In table 1, the frequency of completely sterile plants in the unselected populations Ad 134 and Ad 15 approximates to 25 per cent. as would be expected if a single locus determined the necessity for tripping, with the dominant and recessive alleles equilibrating at equal frequencies.

TABLE 1

Mean pods and seeds set under natural selfing (autofertility)

Line	Mean number of				Per cent. sterile plants	Total plants
	flowers/plant	pods/plant	seeds/plant	seeds/pod		
3Aj 20/8 .	57.5	5.05	9.76	1.93	4.8	21
3Aj 17/10 .	61.8	3.00	5.83	1.94	27.6	29
Ad 134 .	53.7	3.19	5.52	1.73	23.8	21
Ad 15 .	35.3	1.44	3.55	2.47	22.2	9
Ad 15/CF .	36.4	4.60	10.20	2.22	0.0	5
Mean .	48.9	3.46	6.97	2.06		

If in fact a single allelic pair is involved, selection has altered the gene frequency in 3Aj 20/8 but not in 3Aj 17/10.

In genotypes where tripping is not necessary, pollen tube growth is apparently normal. However, if fertilisation does not follow, abscission of the flower occurs. Some increase in length of the ovary begins soon after pollination, but after fertilisation, growth is more rapid presumably as a result of increased hormone production (Lund, 1956). During the inbreeding work on field beans (Rowlands, 1960) large numbers of abortive pods were noticed in some lines, and a similar phenomenon occurred during the investigation of broad beans:

Line	Per cent. of pods which abort
3Aj 20/8 . .	30.8
3Aj 17/10 . .	85.5
Ad 134 . . .	46.8
Ad 15 . . .	48.3
Ad 15/CF . .	25.9
Mean . . .	52.8

An average of 50 per cent. of the pods, which are initially developed, never reach maturity. The growth of pods after self- and cross-pollination is represented in the figure (text-fig. 1). No difference in

TEXT-FIG. 1

normal pod development can be detected between tripped and untripped flowers, and moreover, pod development on selfing is similar to that following cross-pollination. This indicates that pre-fertilisation treatments have no detectable effect on normal pod development. However, pods which subsequently abort but remain attached to the plant are indistinguishable from normal pods for 6 to 8 days after pollination, but their growth rate subsequently slows down and the pods rarely exceed 40 mm. in length. Occasional pods continue growing at a reduced rate, but on closer examination, these are also found to contain aborted ovules (text-fig. 1). Fertilisation must have been successful for any appreciable pod development to take place and hence the sterility has arisen at a post-fertilisation stage.

4. EMBRYOLOGY

The mature embryo sac of broad beans contains a faintly stained egg cell with two deeply staining synergidæ and a faintly stained bipartite central "fusion" nucleus (plate I, fig. 1). During normal embryo development, a four-celled suspensor subtending a four-celled embryo has developed after 4 days (plate I, fig. 2). Four to eight free nuclei constitute the endosperm at this state. After 8 days, the pro-embryo has increased slightly in cell number and the suspensor has begun to differentiate (plate I, figs. 3 and 4). By 12 days after pollination the embryo begins to differentiate and the suspensor becomes vacuolated (plate I, fig. 5). At this stage the ovule is approximately 3-4 mm. long. If, 8-12 days after pollination, fertilisation has not taken place, the egg cell, central fusion nucleus and synergidæ lose their structure and the ovule begins to degenerate (plate I, fig. 6). Following successful fertilisation, a cap of cells begins to form at the lower end of the embryo sac, but occasional ovules develop no further (plate I, fig. 7). However, most ovules develop to the 8-12 day stage, and it is at this stage that many of them subsequently abort (plate I, fig. 8). In the figure some cell development is visible at the lower end of the embryo sac, the suspensor has not become vacuolated and the embryo has not begun to differentiate. Relatively few endosperm nuclei are present and cell proliferation has begun on the wall of the embryo sac (arrowed in fig. 8). Such cell development is characteristic of the somatoplastic sterility described by Brink and Cooper (1939). Ovules degenerating at a later stage are shown in figs. 9 and 10.

5. INBREEDING

Inbreeding studies in broad beans have given very similar results to those previously described for field beans, *i.e.* positive selection for self-fertility has given no marked improvement. Table 2 gives the means for pods and seeds per plant during three generations of inbreeding.

The material recorded in generation 1 was the same as that described in table 1. Values in brackets are the means of plants selected

to provide the next generation. Generation 1 was grown in an insect-proof greenhouse during the spring and summer of 1962, while generation 2 was grown during the autumn and winter using artificial illumination to supplement the natural daylength. This probably accounts for the lower pod and seed set during generation 2. Generation 3 was grown during the spring and summer of 1963 and hence pod and seed set should be comparable with generation 1. No significant increase in self-fertility is present in generation 3. During the inbreeding work, any lines which contained completely sterile

TABLE 2

Mean values per plant for pods and seeds set on self-pollination (autofertility)

Families	Generation 3 (1963)		Generation 2 (1962-63)		Generation 1 (1962)	
	Pods	Seeds	Pods	Seeds	Pods	Seeds
Ad 134 . . .	4.02	7.30	1.90 (3.33)*	2.90 (5.50)	3.19 (7.00)	5.52 (13.32)
Ad 15/CF/2 . .	5.00	10.50	1.70 (4.00)	2.46 (6.00)	4.60 (5.67)	10.20 (13.00)
3Aj 17/10 . . .	3.93	7.50	2.71 (4.33)	5.57 (10.33)	3.00 (5.00)	5.83 (13.00)
3Aj 20/8 . . .	4.56	9.89	1.64 (3.25)	2.77 (7.00)	5.05 (6.25)	9.76 (15.13)
Mean . . .	4.38	8.80	1.99 (3.73)	3.43 (7.21)	3.96 (5.98)	7.83 (13.62)

* Figures in brackets refer to the mean values of plants selected to give the following generation.

plants were discarded and this form of selection was quite successful since in generation 3 no completely self-sterile lines were recorded. The slight increase in pod and seed set in lines Ad 134 and 3Aj 17/10 can therefore be accounted for by the elimination of self-sterile plants, presumably those plants unable to set seed without tripping. This confirms the suggestion that a simple recessive gene determines the necessity for tripping of the flower.

6. DISCUSSION

Although the large-seeded horticultural-type bean diverged from the small-seeded agricultural type at least 1500 years ago (Darlington, 1956) breeding systems of the two types show no large differences. This would either indicate that the same breeding system has developed independently in both types, or what is more likely, that the breeding system has not changed appreciably since the types diverged. It was suggested in a previous paper (Rowlands, 1960) that breakdown of the original outbreeding system resulting from the migration of the species

from its centre of origin and cultivation practices, was followed by zygotic sterility caused by deleterious recessive genes which had previously accumulated under the protection of outbreeding. Moreover, linkage of such deleterious recessive genes into a balanced system would produce a situation where the progression of the species towards further inbreeding was in conflict with the genetic inertia of the system (*cf.* Mather, 1953). The genetic system, in the absence of precise selective forces capable of resolving this conflict, has re-established an intermediate level of hybridity by means of what amounts to a balanced lethal system which has presumably remained relatively unchanged for at least 1500 years.

The similarity of the *V. faba* system with the balanced lethal system operating in some species of *Oenothera* shows that its development is not necessarily dependent on structural changes in the chromosomes. Furthermore, the similarity of this system with the control of self-fertility in *Medicago sativa* (Cooper and Brink, 1940; Cooper, Brink and Albrecht, 1937), *Melilotus officinalis* (Sandal and Johnson, 1953) and *Theobroma cacao* (Knight and Rogers, 1955) suggests that balanced lethal systems may be quite widespread in cultivated plants. As pointed out by Bateman (1954) a *Theobroma* system, gametophytically controlled by both pollen tube and embryo sac, would be indistinguishable in practice from the balanced lethal system characterising some species of *Oenothera*. It would therefore be extremely difficult to establish the true nature of the mechanism determining the level of hybridity in an organism without a full knowledge of its breeding system, the restriction of pollen tube growth by an S-locus mechanism being only a small part of such a breeding system.

Attempts to develop inbred lines and to improve the self-fertility of cultivated species which still exhibit some out-breeding must therefore take into account not only any superficial self-incompatibility system but also the genetic structure of their breeding systems.

7. SUMMARY

1. The behaviour of pod and seed setting in broad beans (horticultural types) is similar to that previously described for field beans (agricultural types).
2. The absence of precise selective forces capable of resolving the conflict between further inbreeding and the genetic inertia of the system has resulted in the establishment of an intermediate level of hybridity which has remained relatively stable for over 1500 years.
3. The similarity of the *V. faba* system to that which undoubtedly operates in *Medicago sativa*, *Melilotus officinalis* and *Theobroma cacao* suggests that such intermediate but stable hybridity levels may be widespread in outbreeding cultivated plants.
4. Attempts to improve the self-fertility of cultivated species which exhibit such intermediate levels of hybridity are unlikely to be successful unless the genetic structure of the whole breeding system is considered.

Plate I

FIG. 1.—Embryo before fertilisation.

cfn = central fusion nucleus

o = weakly staining ovule

s = synergids

FIG. 2.—Developing zygote after 4 days.

FIG. 3.—Developing zygote after 8 days with free endosperm nuclei.

FIG. 4.—Developing zygote after 8 days showing pro-embryo and suspensor.

FIG. 5.—Developing zygote after 12 days showing pro-embryo and vacuolated suspensor.

FIG. 6.—Unfertilised ovule after 12 days.

FIG. 7.—Fertilised ovule but no embryo development after 12 days. c = cap of cells at micropylar end.

FIG. 8.—Degenerating ovule after 18 days. → = cell development characteristic of somatoplastic sterility.

FIG. 9.—Degenerating ovule after 20 days.

FIG. 10.—Degenerating ovule after 24 days.

(Figs. 1 and 4 $\times 960$, remaining figs. $\times 160$).

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