STUDIES ON BRITISH POPPIES
I. SOME OBSERVATIONS ON THE REPRODUCTIVE BIOLOGY OF THE BRITISH SPECIES OF *PAPAVER*

By Stella Rogers

Department of Biology, Queen Elizabeth College, London

Abstract

The breeding mechanisms of the British species of *Papaver* have been re-investigated and it has been confirmed that *P. rhoeas* is self-incompatible and that the other species, *P. dubium, P. lecoqii, P. argemone* and *P. hybridum,* are all self-compatible.

The proportion of self- to cross-fertilized seed in the capsules of the self-compatible species is generally related to aspects of their floral biology such as anther height, flowering period, attractiveness to insect visitors, etc. In *P. dubium,* however, there is also evidence of considerable variation in the degree of inbreeding which is not dependent upon the quantity of self pollen reaching the stigmas, and which would therefore appear to be genetically determined.

Introduction

Bud pollination is a well-known characteristic of the poppies and it was not unreasonable for observers to assume that this resulted in self-fertilization, especially in a genus where there were no obvious features of floral morphology which might be associated with self-sterility. The work of McNaughton & Harper (1960a) has clarified the situation by showing that *Papaver rhoeas* is self-incompatible and *P. dubium, P. lecoqii* and *P. argemone* self-compatible. These workers were, however, primarily concerned with the end result of the reproductive process, that is with the quantity of seed set; it is the object of the present paper to fill in some of the relevant detail.

Methods

Compatibility within and between the British species of *Papaver* was investigated by determining:

(1) the germination of self, cross and hybrid pollen on the stigmas and the penetration of the pollen tubes into the transmitting canal;

(2) the quantities of seed set following self, cross and open pollinations.

Germination of the pollen and subsequent growth of the pollen tubes was observed on cut emasculated flowers kept in a dark incubator at 22° C for 18–24 hours (Lewis 1947), then fixed in acetic alcohol and stained in light green/acid fuchsin.

Self-pollination was either allowed to occur naturally by auto-deposition or was aided by brushing the self pollen over the stigmas to ensure that the amount of seed set was not limited by the amount of pollen reaching the stigmas. Cross-pollinations were carried out twenty-four hours after emasculation and all emasculated and pollinated flowers were enclosed in pollen-proof bags. Open-pollinated flowers were allowed to pollinate naturally and were bagged after the petals had fallen, to avoid loss of seed as the capsules matured and to ensure that the capsules developed under the same conditions as capsules pollinated by the other methods.

Results

1. *Homogamy*

According to Clapham (1962) the species under consideration are all homogamous and bud pollinated. Observations on the self-compatible species *P. dubium, P. lecoqii, P. argemone* and *P. hybridum* show that the self pollen will germinate freely on its own stigmas immediately following anther dehiscence, indicating that the pollen is mature by this stage. Pollen is normally shed while the buds are still in the drooping position, that is, about two

days before the opening of the flower. In *P. rhoeas* there is no immediate germination of the self pollen within the bud after anther dehiscence and in the following forty-eight hours only a few pollen-tubes develop. That this behaviour is not due to the immaturity of the stigmas may be shown by emasculation before anther dehiscence and subsequent pollination with mature cross pollen. The results, summarized in Table 1a, show that the stigmas are already receptive at the time of dehiscence of the anthers. If not pollinated they remain receptive for about a week although seed-setting is not consistent after about five days. The

(a) Stigma receptivity.   TABLE 1. *Papaver rhoeas.*

Days after emasculation		0	1	2	3	4	5	6	7	8
Pollen germination	Number of attempts	21	16	15	13	13	9	4	6	3
	Number of successes	13	14	8	11	12	6	3	5	1
Seed set	Number of attempts	15	14	16	14	10	10	9	5	—
	Number of successes	14	14	16	14	7	6	1	0	—

(b) Pollen Maturity.

		Condition of Anthers		Condition of Pollen	
No. of Flowers examined	Position of Bud	dehisced	undehisced	Starch present	Starch absent
22	Drooping	16	6	22	nil
18	Horizontal	18	nil	18	nil
18	Vertical	18	nil	5	13
36	Opening	36	nil	4 very little	32

time-sequence of pollen development in this species would appear to be rather different from that in the self-compatible species. In the latter, the pollen is starch-free at anther dehiscence and will germinate freely in both 5% and 10% sucrose solutions as well as on the stigmas. It is thus capable of germination prior to the opening of the flower. In *P. rhoeas*, the pollen is still starchy at anther dehiscence and will not germinate in sucrose until the starch has disappeared. This has normally happened by the time the bud opens. The flower is therefore effectively homogamous (Table 1b).
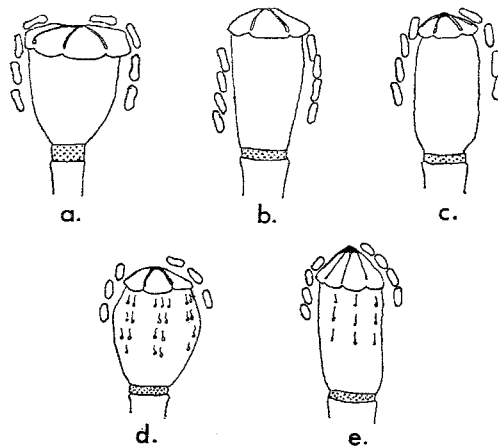

Fig 1. Floral biology in the British Poppies (a) *Papaver rhoeas* (b) *P. dubium* (c) *P. lecoqii* (d) *P. hybridum* (e) *P. argemone.*
The anther level on the right represents the maximum height attained; on the left the normal height.

2. *Anther position*

The significance of anther position in relation to the amount of self pollen reaching the stigmas can be seen from Fig. 1. In *P. rhoeas* and *P. lecoqii* the anthers cover at least one half of the stigmatic disc, and in the latter species the inmost anthers are often firmly wedged between the stigmatic rays so that the pollen comes into intimate contact with the stigmatic papillae at anther dehiscence. *P. argemone* and *P. hybridum* are characterized by having the filaments dilated at the apex and, in the case of the inmost stamens, these filaments may be held by the strong upwardly-directed hairs on the ovary wall, thus maintaining close contact between their anthers and the stigmatic rays. *P. dubium* differs from the preceding species in that the anthers never cover any part of the stigmatic disc and only rarely touch the extreme edge of the stigmatic rays. At anther dehiscence pollen is deposited on the ovary wall, and, since the bud is still then in the drooping position, some may fall on to the ray edges.

3. *Gynoecial structure*

The morphology of the *Papaver* gynoecium has been described by Arber (1938). Pollen is deposited on the stigmatic papillae and upon germination the tubes penetrate between the papillae and into the stigmatic grooves where they grow horizontally until they reach the transmitting canal. This is also lined with papillae, among which the tubes grow vertically downwards into the ovary (Fig. 2a). It follows therefore that pollen tubes arising from grains deposited near the outer edge of the disc must grow appreciably further than those originating from grains nearer the centre of the disc. The tubes are readily identified in the stigmatic grooves and transmitting canal but since the papillae are numerous, it is not possible to follow an individual tube for any distance between them.
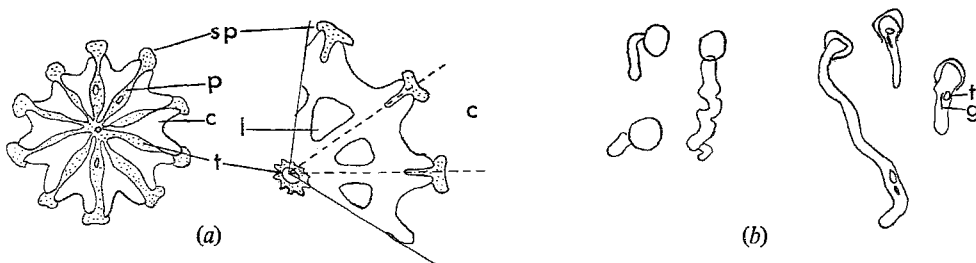

Fig. 2(a). *Papaver rhoeas* stigmatic disc T.S. c = carpel, l = loculus, p = papillae lining stigmatic grooves sp = stigmatic papillae, t = transmitting canal.
(b) *Papaver rhoeas* pollen germination. Left, self pollen tubes after two days on the papillae. Right, pollen germinated in sucrose. t = tube nucleus, g = generative nucleus.

4. *Pollen behaviour*

As already noted, pollen germination in the self-compatible species follows rapidly upon anther dehiscence and the self pollen tubes thus have a considerable time advantage over tubes developing from grains brought by pollinating insects after the flower opens. In *P. argemone*, *P. hybridum* and *P. lecoqii* self pollen tubes may be found along the stigmatic grooves by the time the flower opens and in the latter two species appreciable numbers have also penetrated into the transmitting canal. The relatively small number of self pollen grains reaching the stigmatic papillae in *P. dubium* makes observation of their behaviour more difficult, and their position on the extreme edge of the disc necessitates the pollen tubes traversing the entire length of the stigmatic grooves; thus the time advantage is appreciably less than in those species where the self pollen falls more centrally on the disc. From the above observations it would seem that the degree of inbreeding in *P. dubium* is limited by the position of the stamens and the consequent scarcity of self pollen on the stigmas. This does not appear to be borne out, however, by the results of seed setting experi-

ments described in the next section. In *P. rhoeas*, the development of any self pollen which germinates is arrested at a very early stage. The tubes are often much twisted and distorted and may have swollen ends. There is no evidence of their penetration between the stigmatic papillae or into the stigmatic grooves.

Pollen behaviour in the cultivated Shirley poppy differs from that of wild *P. rhoeas* in that the self tubes grow to a considerable length without obvious distortion or swelling of the tips. There is, however, no observable penetration into the stigmatic grooves and the self-incompatibility barrier is complete. The Mediterranean species *P. apulum* was included inadvertently in one of the trials and, like *P. rhoeas*, it is completely self-sterile (see also McNaughton & Harper 1960a). Its sequence of pollen development resembles that of wild *P. rhoeas* but, as in the Shirley poppy, any self tubes produced are straight and relatively long. Compared with self pollen, unrelated pollen in *P. rhoeas*, the Shirley poppy and *P. apulum* germinates very rapidly on receptive stigmas and numerous tubes may be seen within eighteen hours of pollination. These tubes penetrate straight between the stigmatic papillae and two days after pollination may be found in the transmitting canal. The pollen grains of *P. rhoeas* are binucleate, and when germinated in 5% or 10% sucrose solution the generative nucleus does not divide either within the grain or early in its passage down the pollen tube (Fig. 2b).

5. *Pollen germination of stigmas of other species*

It was found by trial that the pollen of each species will start to germinate freely on the stigmas of all other species. Penetration of the pollen tubes into the stigmatic grooves was not investigated in this experiment. However, long straight tubes were frequently observed penetrating between the papillae and the fact that some interspecific hybrids can be obtained is evidence that the tubes may sometimes achieve the complete journey. Hybrids between *P. rhoeas* and *P. dubium* were included in the trial, though since they were completely male-sterile they could be used only as the female parent. Pollen of all species germinated on the stigma of hybrids having *P. rhoeas* as the female parent. The results for the reciprocal cross are incomplete owing to lack of material.

6. *Seed setting*

The experimental results are summarized in Tables 2 and 3. For the self-compatible species, seed production by self-pollination is expressed as a percentage of the total seed output and as an inbreeding/outbreeding ratio $\dfrac{\text{inbred seed}}{\text{total} - \text{inbred seed}}$.

P. rhoeas. The self-sterility barrier is effectively complete and no evidence was obtained of the pseudo-compatibility reported by Philp (1933) although plants from many different

TABLE 2a. Summary of seed setting experiments.

Pollination Method	P. rhoeas		P. dubium		P. lecoqii		P. argemone	
	% success	Average seed no.	% success	Average seed no.	% success	Average seed no.	% success	Average seed no.
Self	8.1	4	84	591	88	918	73	143
Aided self	5	1	85	455	—	—	64	184
Controlled cross	86	not esti-mated	—	—	—	—	45	212
Open	92	not esti-mated	100	1,434	100	1,695	53	274
Inbred	—	—	5	260	100	395		

TABLE 2b. Inbreeding in the self-compatible species.

	P. dubium (all data)	P. lecoqii	P. argemone
Inbred/total seed %	41	61	64
Inbreeding/outbreeding ratio	0·7:1	1·18:1	1·97:1

The differences between (1) self and aided self (2) controlled cross and open pollinations were not significant at the 5% level.
The differences between self and cross pollinations were significant at the 0·1% level in *P. dubium* and *P. argemone* and at the 1% level in *P. lecoqii.*
Within sample variations are taken into account throughout.

TABLE 3. *Papaver dubium:* Seed set by autodeposition and open pollination.

	Population		
Self pollination	1. Kent	2. E. Suffolk	3. W. Norfolk
Minimum seed no. per capsule	1	31	117
Maximum seed no. per capsule	1,240	1,228	1,922
Av. seed no. per capsule	232	518	1,026
Open pollination			
Av. seed no. per capsule	1,576	1,344	1,827 (10 capsules only)
Inbred/total seed no. %	14·7	38·5	56·15
Inbred/total seed average for species (1,434) %	16·2	36	71·5
Inbreeding/Outbreeding ratio (based on species average for open pollination)	0·19	0·56	2·51

Allowing for within-sample variation, the differences in average self seed number between populations 1 and 2, and 1 and 3 were significant at the 0·1% level and between populations 2 and 3 at the 1% level.

sources were grown. *P. rhoeas* thus resembles *P. nudicaule* in which Fabergé (1943) established that the bud pollination was completely ineffective. As no comparisons could be made between the quantities of seed set following self- and cross-pollinations, estimations of seed numbers were not made for this species. The degree of cross-compatibility is high, indicating that a considerable number of alleles is involved in the incompatibility system. Of 162 controlled cross-pollinations, 139 were successful and most of the failures could be attributed either to the use of old and probably non-viable pollen or to the pollination of very young buds whose stigmas may not have been receptive. All recorded reciprocal crosses were compatible in both directions.

P. dubium. Open-pollinated capsules of this species yielded an average of 1,434 seeds. This is rather less than the number given by Salisbury (1942), but since unset seed was rarely found in the capsules, it may be taken as representing a full seed set. Capsules set by autodeposition contained an average of 591 seeds. This represents 41% of the average number set by open pollination and is a result higher than anticipated in view of the scarcity of self pollen on the stigmatic papillae. That the expected increase in the amount of seed set when self pollen was rubbed over the stigmas was not obtained may be seen from the graph in Fig. 3. The percentage frequency curves for seed numbers following self- and aided self-pollinations are almost identical and the differences between them are not significant statistically. Combining the two sets of data, 30% of the capsules produced by self-pollination contain less than 250 seeds but 7·6% exceed the average number of 1,434 set by open pollination. The very wide range of seed numbers per capsule resulting from self-pollination suggests that individuals may vary in their degree of inbreeding. Unfortunately
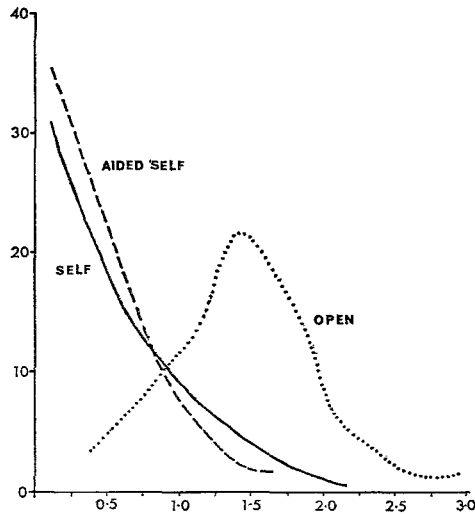
Fig. 3. *Papaver dubium* seed yields resulting from different methods of pollination. Horizontal axis—seed number (in thousands), vertical axis—percentage frequency.

data for replicated self-pollinations on individual plants were not obtained but some evidence that the degree of inbreeding may vary in different populations was found and is summarized in Table 3. The difference in average seed number set by auto-deposition is significant at the 1% level between the east Suffolk and west Norfolk populations, and between these and the Kent population at the 0·1% level. There is also some variation in the average seed set by open pollination, but this is not significant in the case of populations 1 and 2 and it makes little difference to the inbreeding/total seed percentage whether the self seed average is related to the open pollinated seed average of the population or of the species as a whole. In the case of population 3, both the self- and open-pollinated seed averages are considerably above the species averages, though the open-pollinated data were obtained from only ten capsules. Calculated from either open-pollinated average, however, the inbreeding/total seed percentage is obviously high in this population.

    P. lecoqii. The average number of seeds set by self-pollination in this species was 61% of the open-pollinated average (Table 2). This result is appreciably lower than that reported by McNaughton & Harper (1960c) but the data are consistent and would therefore seem to be reliable for plants growing under field, rather than greenhouse, conditions. Flowers which are functionally cleistogamous are not uncommon on otherwise apparently normal plants of this species. The condition results from the failure of the sepals to separate so that the petals do not expand and the stamens remain closely applied to the stigmatic disc. This enforced autonomy is apparently less effective than normal self-pollination. The capsules recorded contained only 38% of the seed number set by open pollination and the difference between the average numbers for self and cleistogamous pollination is significant at the 1% level.

    P. argemone. All methods of pollination yielded very variable results in this species and capsules having a low seed number invariably contained unset seed indicating that fertilization was incomplete. No statistically significant increase in seed set was obtained by rubbing the self pollen over the stigmas—a result anticipated in view of the higher level of the anthers in the flower when compared with *P. dubium*. Controlled cross-pollination gave an increased seed yield and the results obtained did not differ significantly from those obtained by open pollination. Combining the results whose differences were non-significant, the average seed number for open pollination is significantly greater than that for self pollination (at the 0·1% level) and the inbred/total seed percentage calculated on these results is 64%.

The failure of many of the open-pollinated capsules to set fully may be associated with the very short flowering period and the scarcity of insect visits. The flowers open early in the morning and by midday most of the petals and stamens have fallen so that the maximum flowering period is only about six hours. The flowers are not conspicuous when compared with those of *P. rhoeas*, *P. dubium* or *P. lecoqii* and the results of McNaughton & Harper (1960a) for bee flights among plots of the five British *Papaver* species show relatively few visits to *P. argemone*.

DISCUSSION

In the British species of *Papaver*, a store of genetic variability exists within the self-incompatible species *P. rhoeas* and the phenotypic variability of this species in the field is obvious. The self-compatible species exhibit much less phenotypic diversity and their variation pattern can be correlated with their degree of inbreeding. The proportion of self- to cross-fertilized seed in a capsule is determined by:
1) the biology of the flower, including the time of maturity of the pollen and stigmas, the quantity of self pollen deposited on the stigmas, the flowering period and the number of insect visits;
2) the nature of the genetic mechanism controlling the compatibility reaction.

The significance of floral biology and ethological factors is apparent from the studies of McNaughton & Harper and from the present work. The necessary background for an incompatibility system is present in the genus and has been shown to operate effectively in at least three species at the diploid level, *P. rhoeas*, *P. apulum* and *P. nudicaule*. For the last species Fabergé (1943) has shown by the reciprocal nature of the incompatibility that the system is gametophytic rather than a sporophytic one, and it seems reasonable to assume that this applies to all self-incompatible species in the genus. In other genera and families which have been extensively investigated, the same system has been found to operate in all the self-incompatible species studied. Thus pollen control is gametophytic in Rosaceae whereas in Cruciferae all investigated species yield data consistent with sporophytic control (Bateman 1955). Brewbaker (1957) associates late division of the generative nucleus in the pollen grain with gametophytic pollen control in self-incompatible species, and he notes that members of the Papaveraceae have binucleate pollen grains. This is in agreement with the observations made in the present work that division of the generative nucleus does not occur early in the germination of the pollen and supports Fabergé's conclusion that pollen control is gametophytic. On the other hand, Bateman (1955) associates the very early inhibition of self pollen in Cruciferae with sporophytic control and a similar correlation appears to hold in Compositae (Hughes & Babcock 1950, Crowe 1954). Pollen behaviour in the self-incompatible species of *Papaver* is similar to that reported in *Brassica* (Kakizaki 1930), *Capsella* (Riley 1936), *Raphanus* and *Iberis* (Bateman 1955) and indicates that the inhibitory reaction takes place on the surface of the stigmatic rays. It would seem that in the case of *Papaver* this early inhibition is associated with the unusual form of the stigma rather than with the type of pollen control. In other species where a gametophytic incompatibility system operates, the stigmatic surface is separated from the ovary by a style and the inhibitory reaction is generally between the pollen tube and the stylar tissues. In *Papaver* both the stigmatic grooves and transmitting canals are open ducts lined by papillae and, although the pollen tubes grow among the papillae, the same intimate contact between pollen tube and sporophyte cells may not be achieved as when there is a cellular style. Also, the distance traversed by the pollen tubes arising from grains lying centrally in the disc is relatively small so that the inhibition reaction must occur rapidly if it is to be effective.

Self-compatibility in the genus occurs at the diploid, tetraploid and hexaploid levels though the information regarding breeding behaviour at the higher levels of polyploidy is incomplete (Table 4).

In common with other genera which contain both self-incompatible and self-compatible

TABLE 4. Polyploidy and compatibility in the genus *Papaver*.

Species	Compatibility	Chromosome number	Author
P. alpinum	SC	14	Fabergé, 1944
P. apulum	SI	12	Sugiura, 1936
*P. commutatum	?	14	Philp, 1933
P. hybridum	SC	14	Clapham, Tutin & Warburg, 1952
P. rhoeas	SI	14	Lawrence, 1930
P. lecoqii	SC	28	McNaughton, 1958
P. pinnatifidum	—	28	Sugiura, 1940
P. argemone	SC	12	Beale, 1939
		42	Sugiura, 1940
P. dubium	SC	28	Ljungdahl, 1922
		42	Löve & Löve, 1944
P. lapponicum	—	56	Horn, 1938
P. relictum	—	70	Horn, 1938

— no information available

* *P. commutatum* is stated to be naturally cross fertilizing but nevertheless phenotypically uniform and presumably therefore highly homozygous. This requires verification.

species, the pollen behaviour of the British species of *Papaver* does not conform to Lewis & Crowe's rule (1958) that 'in the four possible combinations in crossing self-compatible and self-incompatible species, pollen tube growth is uninhibited except in the cross self-incompatible style—self-compatible pollen, where it is inhibited similarly to self-incompatible germination'. Since these authors argue that all the exceptional genera and species have a recent history of self-compatibility, these observations may be accepted as evidence for the recent self-compatibility in *Papaver*.

The results obtained for *Papaver dubium* are of interest in that they suggest that individuals of this species may vary in their degree of compatibility from almost self-incompatible to fully self-compatible. A rather similar situation is described by Grant (1956) in *Gilia capitata*, where the different taxonomic subspecies show variation in their degree of self-compatibility which can be inversely correlated with their phenotypic variability. Data obtained for *Papaver dubium* indicate that some populations exhibit more phenotypic variability than others in certain characters, though it would not be possible to differentiate discrete taxa on this basis. Variation in the degree of inbreeding has also been reported in both *Petunia* and *Nicotiana* (Mather 1943). In the latter genus, the S-alleles which determine the incompatibility system vary in efficiency and the weakest, when homozygous, will permit the free set of self seed. Such a system may operate in *P. dubium*. The alternative is to suggest a parallel between the hybrid *Nicotiana* × *sanderana* and the species *P. dubium*. The former shows marked pseudo-compatibility in spite of the fact that both its parents are self-incompatible. In Mather's view this pseudo-compatibility results from a breakdown in the efficiency of the S-alleles due to polygenic unbalance in the hybrid. Observation of bivalent formation in the hybrid *P. rhoeas* × *dubium* suggests considerable homology so that at least one self-incompatible species may have been involved in the ancestry of *P. dubium*.

REFERENCES

ARBER, A. (1938) Studies in flower structure, IV. On the gynoecium of *Papaver* and related genera. *Ann. Bot., Lond.* N.S. **2**, 649–664.
BAKER, H. G. (1959). Reproductive methods as factors in speciation in flowering plants. *Cold Spring Harbor Symposium* **24**, 177–190.
BATEMAN, A. J. (1955). Self-incompatibility systems in angiosperms. III. Cruciferae. *Heredity* **9**, 53–68.
BREWBAKER, J. L. (1957). Pollen cytology and self-incompatibility systems in plants. *J. Hered.* **48**, 271–7.

CLAPHAM, A. R. (1962), in CLAPHAM, A. R., TUTIN, T. G. & WARBURG, E. F. *Flora of the British Isles*. Ed. 2. Cambridge.

CROWE, L. K. (1954). Incompatibility in *Cosmos bipinnatus*. *Heredity* **8**, 1–5.

FABERGÉ, A. C. (1943). Genetics of the Scapiflora section of *Papaver*. I. The garden Iceland poppy. *J. Genet.* **44**, 169–193.

GRANT, V. (1956). The genetic structure of races and species in *Gilia*. *Adv. Genet.* **8**, 55–87.

KAKIZAKI, Y. (1930). Studies on the genetics and physiology of self and cross incompatibility in the common cabbage. *Jap. J. Bot.* **5**, 133–208.

LEWIS, D. (1947). Competition and dominance of incompatibility alleles in diploid pollen. *Heredity* **1**, 85–108.

LEWIS, D. & CROWE, L. K. (1958). Unilateral interspecific incompatibility in flowering plants. *Heredity* **12**, 233–256.

McNAUGHTON, I. H. & HARPER, J. L. (1960a). The comparative biology of closely related species living in the same area. I. External breeding barriers between *Papaver* species. *New Phytol.* **59**, 15–26.

McNAUGHTON, I. H. & HARPER, J. L. (1960b). The comparative biology of closely related species living in the same area. II. Aberrant morphology and a virus-like syndrome in hybrids between *Papaver rhoeas* L. and *Papaver dubium* L. *New Phytol.* **59**, 27–41.

McNAUGHTON, I. H. & HARPER, J. L. (1960c). The comparative biology of closely related species living in the same area. III. The nature of barriers isolating sympatric populations of *Papaver dubium* and *P. lecoqii*. *New Phytol.* **59**, 129–137.

MATHER, K. (1943). The genetical structure of populations. *S. E. B. Symposium* **7**, 66–95.

PHILP, J. (1933). The genetics of *Papaver rhoeas* and related forms. *J. Genet.* **28**, 175–203.

RILEY, H. P. (1936). The genetics and physiology of self-sterility in the genus *Capsella*. *Genetics* **21**, 24–39.

SALISBURY, E. J. (1942). *The reproductive capacity of plants*. G. Bell. London.