INCOMPATIBILITY IN BRACKEN

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I. INTRODUCTION

IN bracken, mature male and female sexual organs are present simultaneously on the same prothallus (fig. 1) so that there is no timing device such as has been found in many other ferns, to prevent self-fertilisation. Preliminary tests suggested that an incompatibility system was in operation and the following is an account of experiments carried out to establish its existence and to elucidate its mechanism.

2. MATERIAL

Three populations of bracken were investigated from the localities of Ballochraggan (population B), Killearn (K) and Milngavie (M) in the south of Scotland. Population M differed morphologically from the other two in that the apices of the pinnæ branched to give rise to the so-called "crested" form; also the spores were darker in colour. Spore samples were taken from a *single frond* in each case and prothalli were cultured in petri-dishes on agar containing standard Knop solution. Cultures were kept reasonably clean by plating spores which had been washed several times by centrifugation.

3. METHODS

(i) Preliminary tests

Populations B and K were investigated separately in the following way: several dishes were plated with spores drawn from a spore sample of the particular population under investigation. Some of the prothalli which developed were isolated individually from one of the dishes into phials before they reached sexual maturity. The prothalli in the remaining dishes were maintained so that when they reached maturity they would give a supply of sperm. Sperm suspensions obtained by flooding these dishes with water are referred to as suspensions of "mixed sperm". When the isolated prothalli reached maturity they were tested, some against their own sperm and some against mixed sperm. Each prothallus on being flooded once was examined under the dissecting microscope and only those showing the presence of mature archegonia were taken into account, the criterion for maturity being the massing of sperm in the archegonial mucilage (Wilkie, 1954). The results are given in table 1, and this procedure was carried out in all tests.

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These results indicate that prothalli have a preference for sperm other than their own. It will be noticed that a certain amount of selfing takes place. This is discussed below.

Prothalli were next isolated in pairs and it was found that in each population approximately 50 per cent. of the pairs were compatible,

TABLE 1
*Results of flooding isolated prothalli of two populations
with water and with suspensions of mixed sperm*

Population	Isolated prothalli			
	Flooded with	No. fertilised	Not fertilised	Total treated
B	Water . . .	8	46	54
	Mixed sperm . . .	40	8	48
K	Water . . .	7	44	51
	Mixed sperm . . .	41	8	49

each member of a pair with the other (table 2). In this experiment only those pairs which had mature antheridia and archegonia in both members at the time of flooding were considered. These results suggest a single-gene, two-allele system of incompatibility in each population.

TABLE 2
*Results of flooding isolated pairs of prothalli of each
population with water*

Population	No. of compatible pairs	No. of incompatible pairs	No. of single fertilisations	Total no. of pairs tested
B	17	8	2	27
K	19	12	3	34
M	9	9	0	18

(ii) Tests with clones

For further tests it was necessary to produce clones. This was achieved by sectioning parent prothalli when it was found that regeneration of new prothalli took place from the edges of the segments (fig. 2). These new prothalli were detached, each one into a separate phial and in this way 32 clones were established in all. These were used in series of experiments in which various combinations of clones were tested against each other. The density of sperm suspensions

TABLE 3

*Results of testing clones of population M against each other
in different combinations*

Sperm	Prothalli			
	Clone	Total tested	No. fertilised	Compatibility
Clone 1M . . .	1M	43	3	—
„ 2M . . .		21	1	—
„ 3M . . .		24	2	—
„ 4M . . .		26	1	—
„ 5M . . .		21	0	—
„ 6M . . .		11	9	+
„ 7M . . .		10	0	—
„ 9M . . .		10	1	—
„ 10M . . .		13	11	+
„ 11M . . .		11	1	—
„ 12M . . .		16	15	+
Mixed . . .		37	30	+
Clone 1M . . .	6M	13	12	+
„ 6M . . .		16	1	—
Mixed . . .		17	15	+
Clone 1M . . .	7M	13	1	—
„ 7M . . .		15	0	—
Mixed . . .		10	8	+
Clone 1M . . .	8M	18	1	—
„ 8M . . .		7	1	—
Mixed . . .		18	15	+
Clone 1M . . .	9M	9	0	—
„ 9M . . .		16	1	—
Mixed . . .		9	8	+
Clone 1M . . .	10M	12	11	++
„ 10M . . .		9	1	—
Mixed . . .		10	9	+
Clone 1M . . .	11M	20	0	—
„ 11M . . .		6	0	—
Mixed . . .		12	9	+
Clone 1M . . .	12M	9	8	++
„ 12M . . .		10	1	—
Mixed . . .		17	14	+
Clone 1M . . .	13M	10	10	+
„ 13M . . .		8	0	—
Mixed . . .		9	8	+
Clone 1M . . .	14M	11	1	—
„ 14M . . .		8	0	—
Mixed . . .		9	8	+
Clone 1M . . .	15M	13	11	+
„ 15M . . .		15	1	—
Mixed . . .		8	7	+
Clone 9M . . .	9M	11	1	—
„ 11M . . .		18	1	—
„ 12M . . .		17	16	+
Clone 9M . . .	11M	15	0	—
„ 11M . . .		16	2	—
Clone 6M . . .	6M	13	1	—
„ 12M . . .		17	1	—

— denotes incompatibility when tested both ways, *i.e.* sexes reversed.
++ denotes compatibility when tested both ways.

used in these experiments was standardised by making hæmocytometer counts followed by appropriate dilution although it was found that sperm densities ranging from 50/ml. to 10^4 /ml. gave similar results. The results of these tests are given in tables 3, 4, and 5. Only in those tests in which sperm of clone 6K were used were the results of a doubtful nature. When clone 6K was used as female, on the other hand, there was no doubt as to the results. It appears that the

TABLE 4
Clones of population B tested against each other in different combinations

Sperm	Prothalli			
	Clone	Total tested	No. fertilised	Compatibility
Clone 1B	1B	16	2	—
„ 2B		14	12	+
„ 3B		14	1	—
„ 4B		17	13	+
„ 7B		15	2	—
Clone 1B	2B	27	24	++
„ 2B		21	2	—
Clone 1B	3B	23	2	—
„ 3B		23	3	—
Clone 1B	4B	21	18	++
„ 4B		15	3	—
Clone 1B	5B	16	14	+
„ 5B		15	1	—
Clone 1B	6B	15	13	+
„ 6B		13	1	—
Clone 1B	7B	20	2	—
„ 7B		17	3	—
Clone 2B	2B	16	2	—
„ 3B		12	10	+
„ 4B		13	1	—
„ 7B		12	7	+
Mixed		13	9	+
Clone 3B	3B	13	2	—
„ 7B		22	18	+

mechanism of incompatibility had broken down to a large extent in clone 6K sperm. Otherwise the results listed in the tables indicate the existence of two mating types in each population (the ratios of the two types being 9 : 5 in M, 5 : 4 in K and 3 : 4 in B) the following conditions having been satisfied in each :

- Two clones both compatible with a test clone are incompatible when tested against each other.
- Two clones both incompatible with the test clone are incompatible when tested against each other.

- (c) Two clones, one compatible the other incompatible with the test clone, are compatible when tested against each other. Thus a single-gene, two-allele system may be postulated in each case.

TABLE 5

Clones of population K tested against each other in different combinations

Sperm	Prothalli			
	Clone	Total tested	No. fertilised	Compatibility
Clone 1K	1K {	16	2	—
„ 2K		11	10	+
„ 3K		17	15	+
„ 4K		15	1	—
„ 6K		13	1	—
Clone 1K	2K {	17	15	+++
„ 2K		11	1	—
Mixed		12	10	+
Clone 1K	3K {	18	17	+++
„ 3K		12	2	—
Mixed		13	9	+
Clone 1K	4K {	19	1	---
„ 4K		12	2	—
Mixed		13	12	+
Clone 1K	5K {	15	13	+
„ 5K		8	0	—
Clone 1K	6K {	13	1	---
„ 6K		14	4	?
Clone 1K	7K {	10	9	+
„ 7K		13	1	—
Clone 1K	8K {	17	0	—
„ 8K		14	2	—
Clone 1K	9K {	16	13	+
„ 9K		19	2	—
Clone 2K	4K {	16	14	+
„ 3K		15	10	+
„ 6K		22	10	?
„ 8K		14	1	—
„ 4K		29	3	—
Mixed		14	12	+
Clone 4K	6K {	15	2	—
„ 6K		19	7	?
Mixed		10	8	+

When the two mating types of each population were tested against one another they were found to be cross-compatible in all combinations (table 6) so that, if incompatibility is controlled by a single gene there must be multiple alleles. Also there may be more than two

alleles in each population since spore samples were taken from a single frond.

TABLE 6

Results of testing mating types of populations M, B and K against one another in different combinations

Sperm	Prothalli			
	Clone	Total tested	No. fertilised	Compatibility
Clone 3K . . .	3K	22	5	—
„ 4M . . .		23	21	+
„ 6M . . .		25	24	+
Clone 4K . . .	4K	13	2	—
„ 4M . . .		20	19	+
„ 6M . . .		19	17	+
Clone 3B . . .	3B	11	2	—
„ 4M . . .		11	9	+
„ 6M . . .		10	8	+
„ 2K . . .		15	10	+
„ 4K . . .		14	11	+
Clone 4B . . .	4B	12	2	—
„ 4M . . .		14	12	+
„ 6M . . .		10	7	+
„ 2K . . .		10	9	+
„ 4K . . .		10	8	+

4. SELF-FERTILITY

From the relatively high frequency of selfing (table 7) and of crossing between incompatible types, it would appear that the block to self-fertilisation is not a very strong one.

TABLE 7

Comparison of the number of sporophytes produced by selfing with the number obtained from compatible crosses in populations M, B and K

Population	Prothalli flooded with compatible sperm		Prothalli flooded with their own sperm	
	Total	No. fertilised	Total	No. fertilised
B	195	160 (82 per cent.)	188	23 (13 per cent.)
K	246	208 (85 „)	218	33 (15 „)
M	457	412 (90 „)	297	22 (7 „)

Thus the frequency of selfing is of the order of 16 per cent. in B, 17 per cent. in K and 8 per cent. in M.

5. CYTOLOGY

Developing sporangia on the fronds of populations B, M and K were examined cytologically. The chromosomes in meiotic division showed normal pairing and disjunction.

The chromosomes of the gametophyte were studied in developing antheridia, and those of young sporophytes produced in mass culture, in root tips and frond apices. In all three populations a regular alternation of the haploid ($n = 52$) and diploid number of chromosomes took place (fig. 3) indicating a normal sexual cycle.*

The sporophytes produced as a result of selfing in populations B and K were all of normal morphology (fig. 4). Nearly all were examined cytologically and had the diploid number of chromosomes. Self-fertilisation in population M, however, gave rise to sporophytes all of which were weaklings to a greater or lesser degree. Two distinctly abnormal forms occurred and were the subject of special study. One of these forms (fig. 5) was confined to a single clone and selfing in this clone produced only this form of sporophyte. These sporophytes did not develop fronds but produced a cylindrical shoot variously branched. The branches did not have apical meristems but a vascular system was present. The root, on the other hand, was normal and well developed. There was difficulty in keeping these forms alive and the material available for cytological study was limited. The few successful preparations that were obtained showed these to be diploid (fig. 6).

It was concluded that prothalli of this clone carried one or more recessive genes which acted only in the sporophyte and when in the homozygous state resulted in a semi-lethal condition.

The other abnormal form of sporophyte was not confined to a single clone but occurred in several. The clones which produced this form also produced on selfing sporophytes which, though stunted to various degrees, were of normal morphology and had the diploid number of chromosomes. In this second abnormal form, instead of giving rise to fronds as in fig. 4, the apices of the young shoot developed prothallial tissue. Also roots did not develop (fig. 7). Developing antheridia on these prothallial outgrowths and the apices of the young shoots both showed the haploid number of chromosomes (fig. 8). It was concluded that this type of abnormal sporophyte was of apogamous origin.

Apogamy is a permanent feature in the life-history of some ferns and the elimination of nuclear change in these cases is achieved in one of two ways. Apogamy may be followed by apospory as seen for example in the well-known case of *Asplenium Filix-foemina* and in the present investigation, or unreduced spores may be produced as in *Pteris cretica* for example (Manton, 1950). Apogamy has also been

* The most successful squash preparations were obtained by the method of pretreatment with 8-oxyquinoline as outlined by Tjio and Levan, 1950. The material was immersed in a saturated solution of this substance at 18° C. for four hours, then fixed and stained in acetic-orcein.

produced in some ferns by mechanically preventing fertilisation (Lang, 1929 ; Duncan, 1941) but this was not the case here.

6. DISCUSSION

The results obtained from the incompatibility tests in the three populations suggest a single gene, multiple allele system to be in operation similar to that of the bipolar fungi. To be complete the investigation would require the use of genetic markers segregating and recombining with the incompatibility alleles postulated here. It has not been possible yet to obtain marked strains due mainly to the difficulty of raising sporophytes to the stage of sporulation and the length of time this involves.

Due to the relatively high frequency of selfing which takes place, the incompatibility system would appear to be a weak one. As pointed out by Bateman (1952) one would expect to find in nature incompatibility systems continually arising and in all stages of evolution. In systems too weak for the requirements of a species further selective action would be towards a more effective mechanism and hence towards a modified genetic basis. An example of selection for outcrossing (though not for prevention of selfing) is seen in the studies of Pontecorvo (1953) on the genetics of the homothallic ascomycete *Aspergillus nidulans*. The original strains of this fungus were completely self-compatible, mixed inoculi of two strains giving crossed and selfed asci in proportions usually well below the theoretical maximum for random karyogamy (*i.e.* 1 : 1). Mutants were utilised in numerous crosses and new recombinant strains selected. By continuous selection of recombinants, alleles favouring outcrossing (whether present in the original strains or induced by mutation) were automatically selected. Strains of *Aspergillus nidulans* now exist which, though self-fertile, will give 100 per cent. crossed asci when combined with certain other strains. This behaviour, termed by Pontecorvo (1953) "relative heterothallism", indicates the existence of positive incompatibility, *i.e.* mechanisms of preferential fertilisation not based on the prevention of selfing. It has to be noted that the mechanisms of incompatibility in fungi operate at a level altogether different from those in higher plants, including pteridophytes, as shown here. They operate at the level of karyogamy between nuclei in a common cytoplasm. An exception is seen in cocoa where incompatibility is believed to operate in the embryo sac (see Lewis, 1954).

The condition of partial incompatibility seen in bracken may be adequate in maintaining the necessary interchange of genes and the system may become stabilised at the present evolutionary level. Partial incompatibility of this type may be widespread in ferns where sexual organs develop simultaneously, but to detect it critical tests of the kind described here would have to be carried out.

The incompatibility mechanism appears to act after the time of entry of sperm into the archegonial mucilage since no difference in

Plate I

FIG. 1.—Mature prothallus with male (♂) and female (♀) organs. $\times 30$.

FIG. 2.—Regeneration from the edge of a segment of a prothallus. $\times 20$.

FIG. 3.—The diploid (left) and haploid (right) number of chromosomes of the sporophyte and gametophyte respectively. Left $\times 900$ approx., right $\times 1100$ approx.

Plate II

FIG. 4.—A young sporophyte of normal morphology. $\times 4$.

FIG. 5.—Abnormal sporophyte. Shoot (*s*) does not develop fronds but root (*r*) is well developed. $\times 4$.

FIG. 6.—Squash preparation of root-tip of 5 showing the diploid number of chromosomes. $\times 1000$ approx.

FIG. 7.—Abnormal sporophyte developing prothallial tissue. $\times 8$.

FIG. 8.—Squash preparation of developing antheridia on 7, showing the haploid number of chromosomes and mature spermatocytes. $\times 750$.

4

5

8

6

7

behaviour of compatible and incompatible sperm was detected up to this time. That it acts in the zygote is unlikely since too many sporophytes arise on selfing which are healthy and of normal appearance (at least in populations B and K) for the hypothesis of a lethal condition in the zygote to be tenable. It may be that the barrier arises during the movement of the sperm down through the mucilage in the archegonial neck in analogy to the situation in angiosperms, sperm having to make their way through the mucilage, pollen tubes through the stylar tissue. Lewis (1952) has shown in *Oenothera* that incompatible pollen tubes carry an antigen the antibody to which is produced in the style so that pollen tube growth is inhibited. It is possible that incompatible sperm carry an antigen the antibody to which is present in the mucilage. A very marked change in the mobility of sperm takes place in the mucilage (Wilkie, 1954) and it is feasible that incompatibility operates at this level.

7. SUMMARY

1. Testing for incompatibility involves the production of clones and the isolation of prothalli.

2. Incompatibility is demonstrated for three populations of bracken, M, B and K.

3. Two mating types have been detected in each of the populations, spores being taken from a single plant in each case.

4. The three populations were cross-compatible in all combinations of mating types and a single gene, multiple allele system of incompatibility is postulated.

5. Incompatibility appears to operate between the time of entry of sperm into the mucilage of mature archegonia and their arrival at the egg surface. Incompatibility acting in the zygote is considered unlikely.

6. Incompatibility alleles appear to be relatively weak in their action since selfing was 8, 16 and 17 per cent. respectively in the M, B and K populations.

7. Each population showed a regular alternation of the haploid ($n = 52$) and diploid number of chromosomes in gametophyte and sporophyte respectively, indicating a normal sexual cycle.

8. Selfing in one of the populations gave rise to two abnormal sporophytes, one diploid, the other haploid.

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8. REFERENCES

- BATEMAN, A. J. 1952. Self-incompatibility in Angiosperms. *Heredity*, 6, 285-310.
DUNCAN, R. E. 1941. Apogamy in *Doodia caudata*. *Amer. J. Bot.*, 28, 921-931.
LANG, W. H. 1929. On a variety of *Scolopendrium* that bears sporangia on the prothallus. *Ann. Bot. Lond.*, 43, 353-374.

- LEWIS, D. 1952. Serological reactions of pollen incompatibility substances. *P.R.S.*, B, 140, 127-135.
- LEWIS, D. 1954. Comparative incompatibility in Angiosperms and Fungi. *Advances in Genetics*, 6, 235-285.
- MANTON, I. 1950. *Problems of Cytology and Evolution in the Pteridophyta*. Cambridge.
- PONTECORVO, G. 1953. Genetics of *Aspergillus nidulans*. *Advances in Genetics*, 5, 141-238.
- TJIO, J. H., AND LEVAN, A. 1950. The use of oxyquinoline in chromosome analysis. *Ann. Est. Exp. Aula Dei*, 2, 21-64.
- WILKIE, D. 1954. The movements of spermatozoa of bracken. *Exp. Cell Res.*, 6, 384-391.