A BIOSYSTEMATIC STUDY OF SOME *GLYCERIA* SPECIES IN BRITAIN*

4. BREEDING SYSTEMS, FERTILITY RELATIONSHIPS AND GENERAL DISCUSSION

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INTRODUCTION

In a previous paper of this series (Borrill, 1958) it was shown that in each of the species, *G. declinata* $(2n = 20)$, *G. plicata* $(2n = 40)$ and *G. fluitans* $(2n = 40)$ of section *Glyceria*, morphologically distinct local populations occur in paludal habitats. Five such populations were studied from each species, and shown to be genetically distinct. The results of a study of the breeding systems and fertility relationships of some of these populations (cf. Borrill, 1955) are given in the present paper, followed by a general discussion of the series of four papers.

MATERIAL

The populations were sampled by collecting two seed heads from each of between 10 and 12 plants spread over the area of the population. The limits of individual plants were explored in an attempt to ensure that seed was not derived from a single clone. The seed was pooled, and 24 plants, raised from a bulk lot of this seed, were used as a sample of the potential biotypes present in the population.

The population samples used, to be called "races", are shown in Table 1, attention being concentrated on those of the tetraploid species *G. plicata* and *G. fluitans*. The morphology and habitats of the seed parents were described fully in the third paper of this series (Borrill, 1958).

TABLE 1.

Races used in fertility studies.

Species	Race	Origin of seed parents		
		Locality	Vice-County	
G. declinata	A	Malham Beck	Mid-West York	64
	C	Ulverscroft	Leicester	55
	D	Cadgwith	W. Cornwall	1
	E	Hopedale	Stafford	39
G. plicata	F	Fulbourn	Cambridge	29
	G	Breedon	Leicester	55
	J	Wittering	Northampton	64
G. fluitans	K	Breedon	Leicester	55
	L	Ulverscroft	Leicester	55
	N	Acle	E. Norfolk	27
	O	Acle	E. Norfolk	27

METHODS

The methods of making crosses were based on those described by Jenkin (1924, 1931). The florets to form the female unit were emasculated by hand and enclosed with inflorescences of the pollen parent in glassine paper bags. To study self-fertility, groups of heads were enclosed in pollen-proof bags. These operations were carried out on dry, calm days. The smallness of the florets and anthers made it impossible to emasculate by extracting the anthers with forceps. Anthesis occurs immediately the spikelets have emerged; therefore, as soon as this occurred, the leaf sheaths were split down and the divided portions tied back. Spikelets which had flowered, and those which were too soft to manipulate, were removed. The inflorescence was fastened to a sheet of glass, using plasticine strips, and emasculation was carried out with mounted needles under a low-powered binocular microscope. Any burst anthers and stray pollen were easily seen, and the florets containing them were discarded. After emasculation the leaf sheaths were tied back lightly into place round the culm thus providing mechanical support and reducing the risk of the florets drying out.

After the spikelets shattered on the ripe culms, the bags were opened and the seed spread on a ground-glass plate lit from below. The seed from the bag was divided into two classes, heavy seed, which was judged to be fully developed, and light seed, this category including all seed which, though rounded, was nevertheless below two-thirds of the normal size, also seed which was shrunken and imperfect. A sample of ripe seed collected in the field was used as a standard for the comparison of seed size.

In the emasculated inflorescences, a definite number of florets was used, and the percentage seed set calculated directly. In the selfed- and open-pollinated panicles the total number of florets used was found by counting the number of persistent glume-pairs, which gave the number of spikelets present, and multiplying this number by the mean number of florets per spikelet for the race, obtained from the biometrical data given in paper 3 (Borrill, 1958). A correction factor of 1·5 florets per spikelet was deducted to allow for the proportion of male-sterile apical florets, which often set no viable seed. Rudimentary terminal florets were ignored.

Self- and cross-compatibility were assessed by the number of seeds set, expressed as a percentage of the maximum possible. The seed was sown on a germinator at 25°C. in order to determine its viability.

BREEDING SYSTEMS

Self-fertility

Seed was collected from open-pollinated heads of G. *plicata* and G. *fluitans* to find the capacity for setting seed when no deliberate restriction was placed on the source of pollen available. The viability of this seed was tested and compared with that of seed collected from the original plants in the field. Plants of all three species were selfed, and the results for seed-setting and viability are summarised in Table 2.

In G. *declinata*, selfed seed only was collected from one plant of each race. These plants were highly self-fertile, with a preponderance of heavy seed, and there was little difference between plants in degree of self-fertility. The viability of the seed compared favourably with that of the open-pollinated seed from the original populations.

Seven plants of G. *plicata* were selfed, and each resembled G. *declinata* in being highly self-fertile, their seed-set being as good as that obtained from open pollination. These races have now been maintained by selfing without apparent loss of vigour through three seasons. The behaviour of G. *fluitans* was entirely different, self-fertility being much lower than cross-fertility. The detailed results (Table 3), summarised in Table 2,

TABLE 2.

Summary of seed setting and viability. (For details of G. fluitans see Tables 3 and 5).

Method	Race	No. of selfings crosses or open pollinations	Percentage mean seed-set					Percentage mean germination	
			Heavy	Range	Light	Range	Total	Heavy and light seed	Range
Glyceria declinata Selfings	A	1	62		8			95	
	C	1	67		11			100	
	D	1	64		15			97	
	E	1	83		9			89	
			69*	**62–83**	**11**	**8–15**	**80**	**95**	**89–100**
Open pollination Experimental garden	—	—	—	—	—	—	—	—	
Original populations	—	13	—	—	—	—		**76**	**41–93**
Glyceria plicata Selfings	F	3	77		6·6			93	
	G	2	90		7·0			86	
	J	2	78		4·5			98	
			81	**69–97**	**6**	**4–9**	**87**	**92**	**82–100**
Open pollination Experimental garden	G	1	81		6			97	
	J	2	85		4			81	
			84	**81–86**	**4**	**2–6**	**88**	**83**	**65–97**
Original populations	—	10						78	57–95
Intra-race crosses	F	5	77		12·8			93	
	J	5	75		10·4			96	
			76	**68–81**	**12**	**7–17**	**88**	**95**	**90–100**
Inter-race crosses	F × J	6	74		8·8			87	
	F × G	1	79		9·0			84	
	J × G	2	76		10·0			87	
			75	**70–81**	**9**	**6–12**	**84**	**86**	**80–95**
Glyceria fluitans Selfings	K	4	27		43			55	
	L	3	19		52			51	
	N	2	28		49			39	
	O	2	37		38			67	
			27	**8–58**	**46**	**13–67**	**73**	**54**	**2–90**
Open pollination Experimental garden	K	3	68		22			88	
	O	2	67		13			87	
			67	**65–71**	**19**	**6–23**	**86**	**88**	**87–90**
Original populations	—	12						82	63–95
Intra-race crosses	K	4	60		16			42	
	N	3	59		14			54	
	O	2	71		17			52	
			62	**51–75**	**16**	**12–19**	**78**	**54**	**30–58**
Inter-race crosses	L × N	2	0		0			0	
	L × K	5	2·2		2·4			4·2	
	L × O	3	3·2		7·3			2·6	
	K × N	2	1·0		2·1			0	
	K × O	2	0		0·7			0	
	N × O	3	0		1·8			0	
			1·2	**0–11**	**2·5**	**0–22**	**3·7**	**1·7**	**0–21**

*Figures in heavy type are mean and range percentages.

show that the percentage of heavy seeds is much less on selfing than from open pollination. It is also clear that there is a variation in degree of self-compatibility ; for instance, self-fertility is low in plants *K* 1 and *L* 22 (Table 3), and higher in plants *K* 21 and *O* 218. Table 2 shows clearly that, whilst in G. *declinata* and G. *plicata* self-fertility is uniformly

high, it varies widely in G. *fluitans*. The seed-viability data parallel these results since, in general, the most self-fertile plants, with the largest amount of heavy seed, have the highest germination.

TABLE 3.

G. fluitans *Seed set and viability from selfing and open pollination.*

| Method | Race | Plant No. | No. of spikelets | No. of seeds | | Per cent | | |
| | | | | | | Seed set | | Germination |
				Heavy	Light	Heavy	Light	Heavy plus light seeds
Selfings	K	1	36	27	187	8	57	17
		19	69	126	270	19	41	66
		20	101	308	475	32	49	57
		21	26	126	52	50	25	82
Open pollination		**1**	**70**	**455**	**156**	**68**	**23**	**90**
		8	**98**	**671**	**198**	**71**	**21**	**87**
		13	**48**	**301**	**104**	**65**	**22**	No data
Selfings	L	22	62	60	431	10	65	38
		23	73	158	435	22	55	54
		24	9	24	33	25	37	61
	N	3	35	127	9	41	32	76
		19	26	36	154	16	67	2
	O	1	51	68	259	16	63	44
		18	13	60	13	58	13	90
Open pollination		**13**	**107**	**572**	**184**	**68**	**21**	**88**
		23	**97**	**514**	**50**	**67**	**6**	**87**
Totals:								
Selfed				501	1120	2408	—	—
Open pollinated				**430**	**2513**	**692**		—
Mean per cent:								
Selfed				—	—	—	27·0	45·8	53·6
Open pollinated				—	—	—	**67·8**	**18·6**	**88·0**

Anthesis

The process of anthesis was observed on several occasions, in the greenhouse and out of doors. Anthesis occurs during the morning at a time apparently determined by weather conditions. The terminal spikelets are the first to mature, and maturation then proceeds basipetally node by node. The basal spikelets on each pedicel mature slightly before those above (Plate 8), but anthesis often occurs simultaneously in two or three spikelets on the same pedicel. Within each spikelet, the florets mature strictly from base to apex.

In G. *declinata* (Plate 8), the first sign of anthesis is the slight separation of palea and lemma on a basal floret, followed by the opening of up to six florets in the same spikelet. This process occurs more or less simultaneously in other spikelets. The lemma and palea diverge widely; at the same time the filaments elongate and the rather short, feathery stigmas protrude very slightly. The anthers are small, about 1 mm., standing in the florets at the end of the stiff filaments which equal, or somewhat exceed the length of the lemma. Dehiscence is by two longitudinal slits, many grains being scattered simultaneously, the rest by shaking; the dehisced anthers curl up and shorten appreciably.
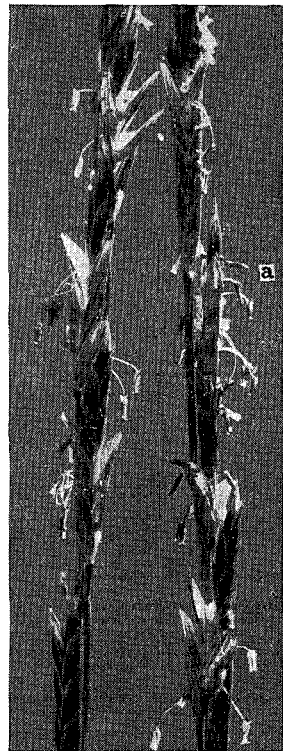
PLATE 8.
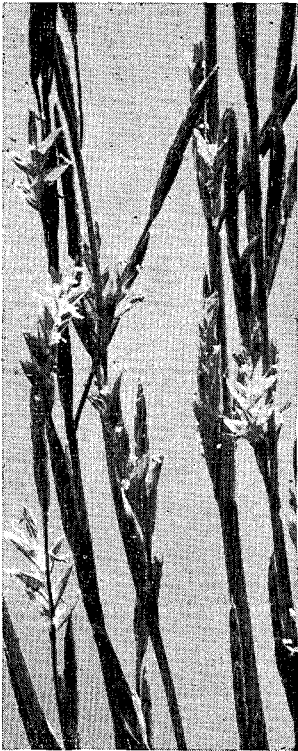
G. declinata   *G. plicata*   *G. fluitans*

Anthesis in *GLYCERIA* species.

Information about spikelet **a** *in text.*

The stigmas are apparently fully receptive when the grains are shed. Whether pollinated or not, the florets remain open for up to ten minutes. In *G. plicata* (Plate 8) the course of anthesis is very similar; the stigmas are a little longer and protude slightly from the angle between palea and lemma. The filaments somewhat exceed the lemma. The anthers are up to 1·25 mm. long and contain larger pollen-grains. After the florets have closed, the shrivelled anthers persist for several days. ˙

In *G. fluitans* (Plate 8) up to 4 florets opened more or less simultaneously in any one spikelet*, and the palea and lemma diverge moderately. Immediately on opening, the filaments elongate rapidly, carrying out the anthers, which are over 2 mm. long. The filaments about equal the lemma, being from 5 to 7 mm. long, and become entirely pendulous, the anthers hanging down below the florets. When the anthers are partly exserted, the stigmas begin to expand; these are long and bushy, protruding conspicuously through the angle between the divergent palea and lemma. Repeated observations led to the conclusion that this species is weakly protandrous. This can be illustrated by successive florets of the marked spikelet in Plate 8. In the uppermost floret the three anthers are emerging and one has dehisced and the stigma protrudes but is not yet expanded. In the floret below, the dehisced anthers hang right down and the stigmas are fully expanded. The florets stayed open for up to 30 minutes, and, when they closed, the stigma and filaments were trapped between lemma and palea.

Discussion

It is clear from these results, that *G. fluitans* differs from the other two species in degree of self-compatibility, and in behaviour at anthesis. To assess the breeding system in natural populations, the chances of self- as against cross-pollination must be estimated. Since a plant's own pollen is more readily available than pollen from adjacent plants, self-fertilisation may occur unless there is a mechanism to prevent it. Because of their high capacity for self-fertilisation, *G. declinata* and *G. plicata* can be regarded as largely inbreeding species, and should be added to the rather small group of inbreeding perennial grasses listed by Beddows (1931).

The possibility that apomixis may occur must not be overlooked, but the stimulus of pollination is necessary for seed to be set, because, when four plants were emasculated and bagged to exclude pollen, no seed was obtained.

G. fluitans can be regarded as mainly an outbreeding species because the plants are largely self-incompatible. Self-fertilisation is nevertheless likely to occur up to the full extent of self-compatibility, as in *Lolium perenne*, in which Griffiths (1950) showed that, on average, about 10 per cent of selfing occurred.

It is interesting that, although the breeding system in grasses is primarily decided by the degree of self-compatibility, it is nevertheless associated with differences in floret morphology and behaviour at anthesis. Inbreeding grasses, which are often annuals, tend to have small anthers which are not well-exserted, whereas outbreeding grasses have large, pendulous anthers, with a lot of pollen and some degree of protandry, or, more rarely, protogyny (Beddows, 1931).

In the third paper of this series (Borrill, 1958) it was shown that *G. fluitans* was more variable than *G. declinata* or *G. plicata*. The present results suggest that this is associated with the breeding systems, the outbreeding species, *G. fluitans*, being the most variable.

In circumstances where adaptability, and consequently high variability, is at a selective premium, self-incompatibility has an obvious advantage. Large anthers may have the advantage of distributing the pollen more efficiently, though the total amount of pollen

* According to Arber (1934) anthesis occurs in each spikelet as it emerges from the leaf sheath. The author's observations are that in all three species anthesis often, but not always, occurs in the basal florets of a spikelet just after emergence, depending on the weather conditions.

per plant may be no more than that produced by a plant with small anthers. Protandry, which often exerts no effective control over the breeding system, may merely be a mechanical consequence of rapid elongation in the long filaments associated with the exsertion of large pendulous anthers.

FERTILITY RELATIONSHIPS

Interspecific crosses

Six crosses between diploid and tetraploid species were made, and four crosses between tetraploid and tetraploid, using large plants dug up from the field. Crosses between species were attempted in both directions, but reciprocal crosses between the same individuals were not practicable. The data are shown in Table 4. Crosses of diploid and tetraploid were completely unsuccessful, as no trace of seed development was found.

When the seed from the tetraploid crosses was sown on filter-paper pads on a constant temperature germinator, no seedlings were obtained and all the seeds were subsequently dissected. From three crosses of G. fluitans and G. plicata, 15 light seeds were obtained (11 per cent seed-set). In one seed (cross No. 7), containing an imperfect embryo and some endosperm, the radicle began to grow. Two seeds of cross No. 8 produced a weak radicle and hypocotyl. The other seeds contained the remains of endosperm, or only watery fluid. From one cross of G. plicata and G. fluitans, 25 seeds were obtained (58 per cent seed-set); these were of approximately normal length, but all were empty except two which contained traces of what was probably endosperm, and none germinated.

TABLE 4.
Interspecific crosses in Glyceria species.

No.	Crosses			No. of florets emasculated	No. of caryopses	
					Heavy	Light
1	G. declinata ♀ (2n = 20) H.446*	×	G. fluitans ♂ (2n = 40) H.440	37	—	—
2	G. fluitans ♀ (2n = 40) H.439	×	G. declinata ♂ (2n = 20) H.413	51	—	—
3	H.435		H.413	62	—	—
4	G. declinata ♀ (2n = 20) H.413	×	G. plicata ♂ (2n = 40) H.423	35	—	—
5	G. plicata ♀ (2n = 40) H.423	×	G. declinata ♂ (2n = 20) H.446	71	—	—
6	H.441		—	60	—	—
7	G. fluitans ♀ (2n = 40) H.439	×	G. plicata ♂ (2n = 40) —	49	—	2
8	H.439		H.423	56	—	13
9	H.437		—	23	—	—
10	G. plicata ♀ (2n = 40) H.442	×	G. fluitans ♂ (2n = 40) H.440	43	—	25

* Herbarium citations given in the following abbreviated form: H.446 = Specimen No. 54446 in the Herbarium of the University College of Leicester.

In all the crosses, conditions for pollination were reasonably good, and the entirely negative results of crossing diploid and tetraploids may have some value in that, although the flowering times of the species coincide, triploid hybrids do not appear to occur in the field where the parents grow together. Both Hubbard (1954) and Lambert (1949) found plants which were considered on morphological grounds to be hybrids between *G. fluitans* and *G. declinata,* but the crucial test of the existence of such hybrids is the demonstration of their triploidy. All the doubtful sterile plants found in the present study, whether growing with their supposed parents or not, were examined cytologically and as all had $2n = 40$ they must be regarded as specimens of *G. × pedicellata.*

The result of crossing the tetraploids differs according to the direction of the cross, since, with the outbreeding species *G. fluitans* as pollen parent, a good percentage of normal-sized but empty seeds was obtained. With the inbreeding species *G. plicata* as pollen parent, a smaller number of shrunken seeds was obtained of which three were partially viable. A similar result was obtained by Jenkin (1954a) in crosses between species of *Lolium,* differing in breeding system, where the general picture suggested that " in the self-pollinating annual species the maternal tissues of the ovaries respond readily to pollination by wind-pollinating types, so that the caryopsis integuments reach full development, even though their contents may be very meagre when the seed is mature. The response of the maternal tissues of the ovaries of *Lolium perenne* to the pollen of the self-pollinating species also follows a general pattern. In this case these tissues do not give a full response and develop only *pari passu* with the embryo and/or endosperm."

The hybrid between *G. fluitans* and *G. plicata, G. × pedicellata,* occurs abundantly in nature. The result of crossing these species suggests that the hybrid is not easy to produce, and that the chance of success is greater with the inbreeding species, *G. plicata,* as the pollen parent.

INTRASPECIFIC CROSSES

Inter- and intra-race crosses were made in *G. plicata* and *G. fluitans,* and, because a wide variation in degree of cross-compatibility occurs, especially in outbreeding species, according to the genotype used and the environmental conditions (Jenkin, 1931, Nilsson 1934), the same plants of *G. fluitans* were used in both types of cross, which were carried out as far as possible on the same day.

In *G. plicata,* a number of reciprocal and direct crosses were made within and between races. The results, which are summarised in Table 2, show that the plants were highly cross-compatible both within and between populations, since in both cases the percentage seed-set was as good as that on open-pollinated inflorescences. The seed viability was also very high.

In *G. fluitans* one reciprocal and two direct crosses were carried out within Race *K,* three crosses within Race *N,* and two within Race *O.* The results are summarised in Table 2, from which it appears that the seed-set approaches that in open-pollinated inflorescences, the bulk of the seed being heavy. The viability, however, was lower than that of seed from open pollination. It is worth noting that the range in viability of seed from these crosses was less than that of seed from selfing.

Four reciprocal and nine direct crosses were made between plants from different races of *G. fluitans.* The results are shown in detail in Table 5 and summarised in Table 2. No seed was obtained in the majority of cases. Heavy seed was produced in two crosses only, *L* 17 × *K* 21 and *K* 8 × *O* 4. Four seedlings were obtained from nine heavy seeds in the former cross, and one from four heavy seeds in the latter. No light seeds germinated. The four F_1 plants of *L* 17 × *K* 21 reached maturity, and were phenotypically intermediate between the parents. Further studies of these plants are in progress.

The results suggest that plants within each race of G. *fluitans* were cross-compatible, whereas plants from different races were largely incompatible. In this respect the behaviour of G. *fluitans* is entirely different from that of G. *plicata*.

TABLE 5.

G. fluitans *seed setting and viability in inter-race crosses.*

Race	Cross: Plant Nos.	Emascu- lated florets	No. of seeds set		Percentage seed-set		No. of seeds sown	Germination		Empty* seeds
			Heavy	Light	Heavy	Light		Number	Per cent	
L × N										
	17 × 12	37	0	0	—	—-	—	—	—	—
	21 × 16	42	0	0	—	—	—	—	—	—
L × K										
	17 × 21	84	9	10	10.7	11·9	19	4	21·1	13 + 2†
Reciprocal {	18 × 22	26	0	0	—	—	—	—	—	—
{	22 × 18	31	0	0	—	—	—	—	—	—
	19 × 23	29	0	0	—	—-	—	—	—	—
	20 × 24	35	0	0	—	—-	—	—	—	—
L × O										
Reciprocal {	8 × 4	41	4	9	9·75	21·9	13	1	7·69	12
{	4 × 8	45	0	0	—	—	—	—	—	—
	5 × 1	40	0	0	—	—	—	—	—	—
K × N										
Reciprocal {	9 × 4	46	0	2	—	4.34	2	—	—	2
{	4 × 9	39	0	0	—	—	—	—	—	—
K × O										
Reciprocal {	13 × 8	64	0	1	—	1·56	1	—	—	1
{	8 × 13	29	0	0	—	—	—	—	—	—
N × O										
	20 × 24	54	0	2	—	3·70	2	—	—	2
	19 × 23	25	0	0	—	—	—	—	—	—
	22 × 18	42	0	0	—	—	—	—	—	—
Total		709	13	24	—	—	37	5	—	32
Mean percentage		—	—	—	1.20	2·55	—	—	1·69	—

* Apparently normal seeds, but contained watery fluid only.

† Two seeds with some endosperm present, no embryo.

In assessing the information obtained about fertility relationships, it is clear that, although only a limited number of selfings and crosses were carried out, the results are consistent, which suggests that, from the behaviour of the sample of plants studied, legitimate inferences can be drawn concerning the behaviour of the populations as a whole.

CONCLUSIONS

1. The results of selfing plants of G. *declinata*, G. *plicata* and G. *fluitans* showed that the first two species were highly self-fertile, while the plants of G. *fluitans* were to various extents self-sterile. From this it appears that G. *declinata* and G. *plicata* are generally inbreeding, and G. *fluitans* generally outbreeding. Associated with this, characteristic differences were observed in behaviour at anthesis, and it has been shown that G. *fluitans* has the greatest variability (Borrill, 1958).

2.   Attempts were made to cross *G. declinata* (diploid) with the tetraploids, and to cross the tetraploids. Crosses were tried in both directions. No trace of seed development was observed in diploid-tetraploid crosses. In crosses between the tetraploids, although no seedling plants were obtained, seed development differed according to the direction of the cross, and the chance of success seemed greater with *G. plicata*, the inbreeding species, as pollen parent.

3.   Inter- and intra-race crosses were made in *G. plicata* and *G. fluitans*. In the first species, all the plants used were highly cross-compatible both within and between races ; whilst in *G. fluitans* although plants within a race were compatible, those from different races were generally cross-incompatible.

GENERAL DISCUSSION

The object of this discussion is to consider briefly the evolutionary relationships of the British species of *Glyceria* and the microevolution of local populations, in the light of the information presented in this series of papers.

The three sympatric species, *G. declinata* ($2n = 20$), *G. plicata* ($2n = 40$) and *G. fluitans* ($2n = 40$), appear to be genetically isolated, because gene exchange does not occur between the diploid and the tetraploids, or between the tetraploids, since their hybrid *G. × pedicellata* is completely sterile. They are therefore coenospecies in the sense of Clausen (1951).

Although the affinities of these species have not been fully established, some evidence has been obtained. Cytological analysis reveals some affinity between at least one genome of *G. plicata* and *G. fluitans*, and it is probable that the species have a common ancestor, which may be *G. declinata*. Several lines of study suggest that *G. declinata* and *G. plicata* are closely related, a conclusion different from that reached by Lambert (1949). These are :

1.   The species are morphologically very similar and have the same breeding system ; as Stebbins (1950) has pointed out, tetraploid grasses generally have the same breeding system as closely related diploids.

2.   The occurrence of some quadrivalents in *G. plicata* together with other evidence, suggests that this species may be an autopolyploid based on *G. declinata*. There are, however, some morphological differences between the species, for instance in stolon formation, the genes for which may have arisen by mutation after the formation of the tetraploid species, or may have been contributed by another, as yet unknown, diploid parent.

3.   *G. declinata* and *G. plicata* have a similar geographical distribution in Europe*, whereas *G. fluitans* is an outbreeding species widely distributed in north temperate regions.

The species of section *Glyceria* occur in Britain as small local populations in paludal habitats. When some of these were studied experimentally, it was found that their distinctive morphological features were genetically determined; they nevertheless appeared to have no ecologically adaptive significance. The situation can be contrasted with that demonstrated by Gregor (1944) in *Plantago maritima* populations inhabiting a range of habitats in coastal mud flats, and having a discontinuous distribution. These local populations could be arranged in ecoclines, and merited the status of ecotypes – " the product arising as a result of the genotypic response of a species to a particular type of habitat " (Turesson 1922).

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* *G. declinata* is apparently introduced in California and Nevada according to Church (1949).

The *Glyceria* populations studied are not ecotypes, but this does not mean that ecotypes do not occur in the group, since there is at least one in *G. fluitans*, a form with a rather stiff type of leaf, and a simple spiciform inflorescence, which is apparently the result of the parallel response of different biotypes to acid habitats. In the other species the occurrence of ecotypes is less certain. Lack of adaptive significance in the phenotypic differentiation of *Glyceria* populations suggests that the selection pressure is not very effective on the features studied; this may be in part due to the fact that paludal habitats have a certain uniformity. The basic requirement for survival appears to be that the species should be physiologically well adapted to seedling establishment and subsequent growth in wet soil. This means that they have a competitive advantage as colonisers of paludal places, which are often disturbed or open habitats, considerable morphological variation occurring independently of this physiological adaptation.

An examination of the genetic structure of the population may help in understanding how morphological differences have arisen. The variability of inbreeding species is small, compared with that of outbreeders, which carry a large reserve of variability in the heterozygous state. This has been shown experimentally by Gregor & Lang (1950) in *Plantago*, by Baker (1953) in *Armeria*, by Cooper (1954) in *Lolium*, as well as in *Glyceria* (Borrill, 1958). Breeding systems in nature are not mutually exclusive, however. For instance, Griffiths (1950), using genetic markers, showed that, in the generally outbreeding species, *Lolium perenne*, on an average, about 10 per cent self-fertilisation could occur. Similarly in inbreeders some outcrossing may occur, up to 1 per cent, for instance, in self-fertilising cereals (Hays & Immer, 1942).

What is the effect of small population size on species differing in variability to the extent that *G. declinata* and *G. plicata* differ from *G. fluitans* ? In inbreeders each population founded by one or more individuals is likely to differ morphologically from other populations, and, provided these are not contiguous, self-fertilisation *per se* will eliminate introgression and therefore reproductive isolation will be complete. This is probably the main reason why in these inbreeding species we find that distinct sub-groups, each with little variability, are easily recognised.

In outbreeders, such as *G. fluitans*, small population-size is likely to have a considerable effect independent of the selection pressure, because :

1. Local populations are likely to be morphologically different owing to their origin from small, unrepresentative biotype-samples ; for instance, populations may arise from the chance introduction of a few seeds.

2. The small number of individuals will result in a change of genetic environment (Mayr 1954) which may change considerably the selective advantage of genes. Furthermore, there will be an increase in the number of homozygotes which are exposed to selection, and, under these circumstances, genes will be favoured which are especially viable in the homozygous state. Thus, in a small population, the genes which have the greater viability may not be the same as those in the population from which the founder-plants came; they will be selected for, and phenotypic changes will occur, which may be rapid, even though the environment may be similar to that from which the founder-plants originated.

3. The occurrence of genetic drift (Wright, 1931, 1940) resulting in the random fixation of morphological markers.

Genetic mechanisms therefore exist whereby morphological population differentiation, of no apparent ecological value, can take place in *G. fluitans*. The situation in this species is comparable with that in *Iris virginica* (Anderson, 1936), a species which occurs in isolated small colonies in paludal habitats. The morphological characteristics of the populations are unrelated to the type of habitat, and, furthermore, the differences between populations

are not proportional to the distances separating them; as Dobzhansky (1937) has pointed out, this is a good example of the occurrence of genetic drift. It can be surmised that some of these genetic changes, associated with small population size, have operated in *G. fluitans*.

Evidence has been obtained to suggest that there is a reduction in the cross-compatibility of some *G. fluitans* populations. Examples of cross-incompatibility within a species, not associated with differences in chromosome number, have been described in *Elymus glaucus* (Snyder, 1951), in *Lolium perenne* (Jenkin, 1954b), and in *Oryza sativa* (Tereo & Midusima, 1939). In the last species there was a wide range of variation in degree of sterility amongst the F_2 progeny of partially sterile F_1 hybrids, indicating polygenic inheritance of incompatibility factors.

In outbreeding species considerable spatial isolation is required to prevent gene-flow; for instance, in *Lolium perenne*, isolation must exceed 200 yards from the nearest large source of pollen to reduce contamination below 4 per cent (Griffiths 1950). Cross-incompatibility could therefore be an important factor in genetic isolation, but there are certain difficulties in accounting for the origin of incompatibility between populations of *G. fluitans*. Cross-incompatibility must occur at random by mutation or chromosomal re-arrangement, and would spread only if selectively advantageous, that is, if gene exchange between populations reduced their fitness to their habitats. The evidence suggests, however, that the phenotypic differences between populations are not linked to physiological characters of adaptive value, and, if the incompatibility factors are selectively neutral, there is no apparent reason why they should spread in the population. Further work is required on incompatibility between local populations in *G. fluitans*.

Some paludal habitats are quite stable, while others, especially those by moving water, will be unstable. It is probable that small populations of *G. fluitans*, isolated for a short time, may change appreciably, since the rate of microevolution is more rapid in small populations than in large ones (Mayr 1954), whether the differentiation which occurs is adaptive, or is due to changes in genetic background or to drift. The origin of small populations is accompanied by a reduction in variability, and those which, thus depleted, retain the capacity for further change must be highly heterozygous. The outbreeder, *G. fluitans*, should possess this capacity which is probably reflected in the great geographical range of the species.

In this connection, *G. fluitans* var. *islandica* Löve is of interest, because small local populations similar in their short lemmas have been found in Britain. Löve (1951) suggests that the morphological features of the variety " are the result of long isolation of the Icelandic populations, together with strong selection by climatic factors during at least the last glaciation " ; but it is also possible that var. *islandica* is entirely derived from the accidental introduction of a few plants with short lemmas, especially in view of the fact that, according to Löve, the varietal characteristics are apparently not linked to any feature of physiologically adaptive value.

The taxonomic status of local populations of *Glyceria* must be considered. They are not ecotypes, and there is as yet insufficient evidence to call those of *G. fluitans* ecospecies; in any case the tendency to emphasise incompatibility-barriers assessed experimentally has certain disadvantages, since what matters from the standpoint of microevolution is the occurrence of reproductive isolation in nature (Cain, 1953) and, as has been indicated, this may occur in several ways. The Icelandic form of *G. fluitans* has been accorded varietal status by Löve (1951). Many local races of *Glyceria* species in Britain are as distinct as this, and could be given varietal names. However, in my view, it is better, while noting that these forms exist, to await a more extensive study of the group before proposing additional names.

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