

NATURAL HYBRIDIZATION OF *AGROSTIS TENUIS* SIBTH. AND *A. STOLONIFERA* L.

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(With 8 figures in the text)

SUMMARY

There seems to be little barrier to natural hybridization between *Agrostis tenuis* Sibth. and *A. stolonifera* L. The F_1 hybrid is vigorous: but it is relatively sterile.

The analysis of natural mixed populations of these two species from an old grazing meadow by means of a morphological index and by pollen fertilities shows that over a large central area of the meadow, plants indistinguishable from F_1 hybrids are very abundant, to the virtual exclusion of the parental species which would be expected in such a habitat. A more elaborate morphological and cytological analysis confirms this, but suggests that there is also a small percentage of plants belonging to F_2 and backcross generations.

The success of the hybrid is due to its growth habit, which is better adapted to grazing conditions than that of either parent. Under grazing conditions reproductive sterility is no disadvantage.

The hybrid populations are made up of a large number of different individual F_1 hybrid plants, and cannot be due to the spread of one hybrid plant throughout the meadow, but to the repeated formation of the hybrid. Preliminary examination of other habitats suggests that the hybrid is more common than previously suspected.

Discussion from an ecological standpoint suggests that the success of natural sterile hybrids depends on the possession of strong powers of vegetative propagation originally derived from the parental species. For this reason *Agrostis tenuis* \times *stolonifera* is particularly successful. There are, however, other contributory factors.

Discussion from a genetical standpoint suggests that since the hybrid is sterile and subsequent generations are uncommon, the two species are likely to remain quite distinct, but that gene flow between them cannot be ruled out without further investigation.

INTRODUCTION

The taxonomy of *Agrostis tenuis* Sibth. and *Agrostis stolonifera* L. has always been difficult. Many early authors such as Bentham and Hooker (1912) grouped the two species under *Agrostis alba* L. and although all later authors recognize the two species, many refer to intermediate types, probably of hybrid origin. Philipson (1937) in his excellent account of the genus, however, did not report any such hybrids, though Fouillade (1932) in France described presumed hybrid types very critically. It remained for Davies (1953) to produce the hybrid experimentally. He showed that it could be produced without any difficulty under experimental conditions. In crosses between *A.*

tenuis and *A. stolonifera* a mean of 30.5 seeds per panicle was formed (as opposed to 3.8 on selfing): in the reciprocal crosses the mean was 177.6 (as opposed to 5.5 on selfing). These results were obtained by bagging together one plant of each species. The flowering times of the two species differ very little. Under natural conditions, if the two species are growing together it seems that there would be little barrier to the formation of hybrids.

The hybrids showed considerable sterility. The seed fertility was about 25 per cent of that of the parents when allowed to be open pollinated and about 2 per cent of that of the parents when intercrossed. The parental species both have the chromosome number $2n = 28$, but cytological analysis of these same hybrids by Jones (1953), (1956) showed considerable disturbance of meiosis and the formation of a mean of 7.5 univalents per pollen mother cell. Pollen fertilities of the hybrids ranged between 40 per cent and 1 per cent with a mean at 13.6 per cent.

Details concerning the artificial hybrids are therefore fairly complete, but the situation requires further examination in view of the somewhat conflicting evidence. It would be reasonable to expect that owing to the ease of intercrossing of the two parents, the hybrid would occur naturally despite its sterility. Fouillade (1932) reports extensive areas of the hybrid near Tonnay Charente in France and suggests that it can survive by vegetative growth, and that under certain conditions it may do better than either parent. This very interesting comment alone justifies further investigation.

POPULATIONS IN PORT MEADOW

In the course of work on the genecology of *Agrostis tenuis*, many natural populations were examined and tiller samples taken into cultivation. In several populations many plants collected as *A. tenuis* turned out subsequently to be intermediate between *A. tenuis* and *A. stolonifera*. One population sample in particular for Port Meadow, near Oxford, was found to be composed almost entirely of intermediates. This locality has been the subject of further, more intensive study.

Port Meadow (described by Baker, 1937) is a large expanse of alluvial grazing meadow on which the commoners of Oxford have had the right of grazing since time immemorial. It is mentioned as such in the Domesday Book. The only record of its not being grazed is during the Civil War, when a special order was necessary to put the meadow up for hay to supply fodder for the Royalist horses. This confirms that at other times it was continuously and heavily grazed by the commoners. The vegetation is that typical of base-rich grazed meadow land. The lower, damper parts are dominated by *Festuca pratensis*, *Agrostis stolonifera* and *Glyceria fluitans*; the upper, drier parts by *Lolium perenne*. The damper regions coincide with a 4 ft. difference in level. These are regularly flooded; the drier regions rarely are. The middle regions would seem to be a normal habitat for *A. stolonifera* and the driest regions for *A. tenuis*.

The earlier sampling was from region B, while later samples were from A, C and D (see map). Region A is the driest part of the meadow, and region D the wettest of those where *A. stolonifera* is found in any quantity. B and C are intermediate. The distances between sites is about 200 m. Samples A, C and D consisted of about sixty tillers taken at random within an area about 50 m. in diameter. Sample B was similar but was taken over a wider area. All were subsequently grown as spaced plants in garden conditions. Sample B was grown in the gardens of the Welsh Plant Breeding Station at Aberystwyth; A, C and D were grown in the garden of the Department of Agricultural Botany at Bangor.

Only vegetative sampling will bring to light the real composition of such populations as those of grasses which are predominately vegetatively reproducing. Without such sampling it is unlikely that the curious nature of the *Agrostis* populations of Port Meadow would have been realized. The possible errors from such sampling, the possibility

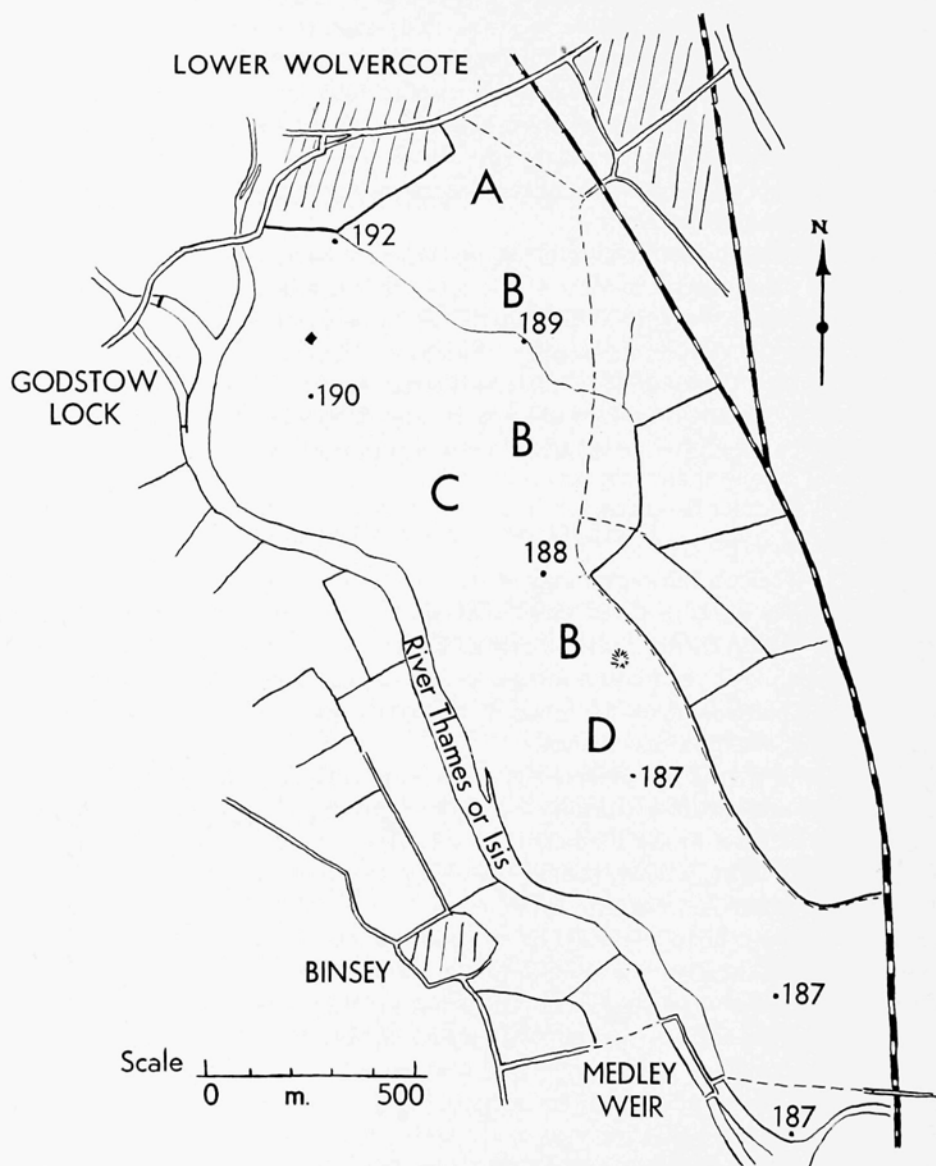


Fig. 1. Sketch map of Port Meadow, Oxford. Spot heights are given in feet. Letters show sampling sites (see text).

that some of the differences between plants are residual effects of the environment and are therefore phenotypic, are small. Under cultivation the plants soon grow away from the original tiller and achieve the growth form and characteristics due to their inherent genetic make-up. This is the experience of other workers (Heisey, 1940) and will be discussed more fully elsewhere.

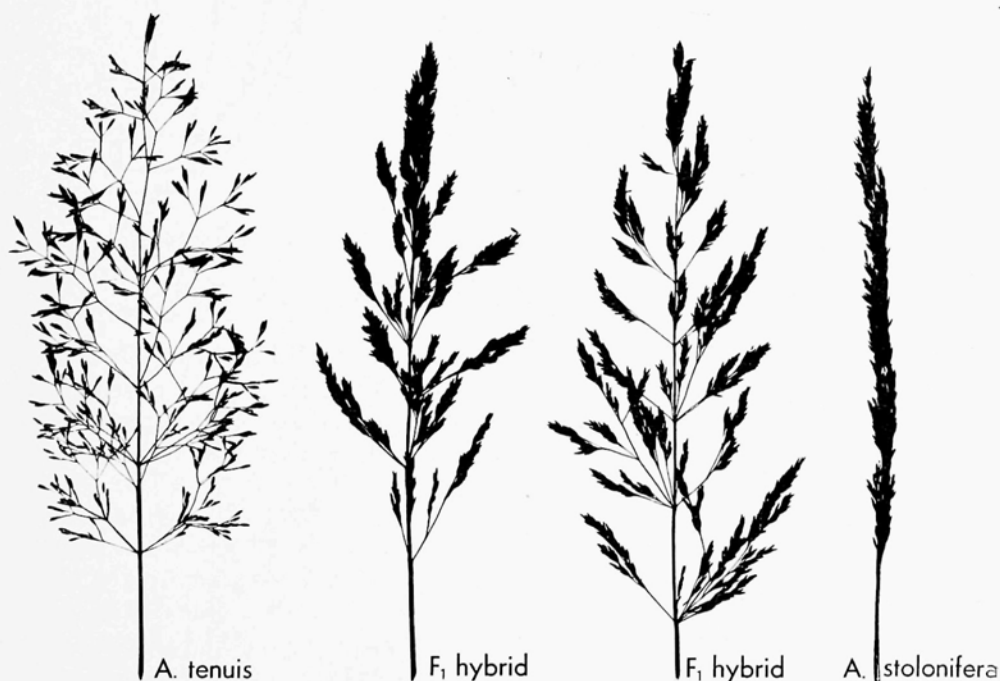
GENERAL ANALYSIS OF POPULATIONS

The four populations have been analysed on morphological criteria. The characters used were those commonly used by systematists to differentiate the two species. None of the characters taken could be treated metrically and a simpler method of analysis had to be adopted. This was unfortunate since with metrical methods degrees of back-crossing and introgression are easily determined, as in *Salvia* (Epling, 1947) and *Crataegus* (Bradshaw, 1953).

Table 1. *Characters used to distinguish the two Agrostis species*

	<i>A. tenuis</i>	<i>A. stolonifera</i>
(a) Growth habit	Bushy and rhizomatous	Spreading and stoloniferous
(b) Ligule of vegetative shoots	Less than half as long as broad and square	Longer than broad and pointed
(c) Panicle	Pyramidal, open in fruit	Cylindrical, closed in fruit
(d) Pedicels and base of spikelet	Almost smooth	Scabrid
(e) Lemma	3 nerved	5 nerved

For each character plants were classified as either *A. tenuis*, *A. stolonifera*, exact intermediates, or other types. The last class was used when the character was intermediate but tending to that of either *A. tenuis* or *A. stolonifera*. To arrive at an overall assessment of each plant these characters were combined together.

Fig. 2. Diagnostic characters of *Agrostis tenuis*, *A. stolonifera* and F_1 hybrid: panicles after flowering.

For each character, plants resembling *A. tenuis* scored 0, those resembling *A. stolonifera* 4, exact intermediate 2, intermediates tending to *A. stolonifera* 3, intermediates tending to *A. tenuis* 1. The scores for each of the five characters used were summed and the plants given an assessment as follows:

<i>A. tenuis</i>	0-3
Exact intermediates	8-12
<i>A. stolonifera</i>	17-20

This numerical method is similar to that described by Anderson (1936) and subsequently used by many other authors. But most authors have been content to assess different characters with scores differing in range. If many different grades of a given character could be distinguished, the score would have a large range; this would mean that such characters would contribute disproportionately to the final index. In assessing

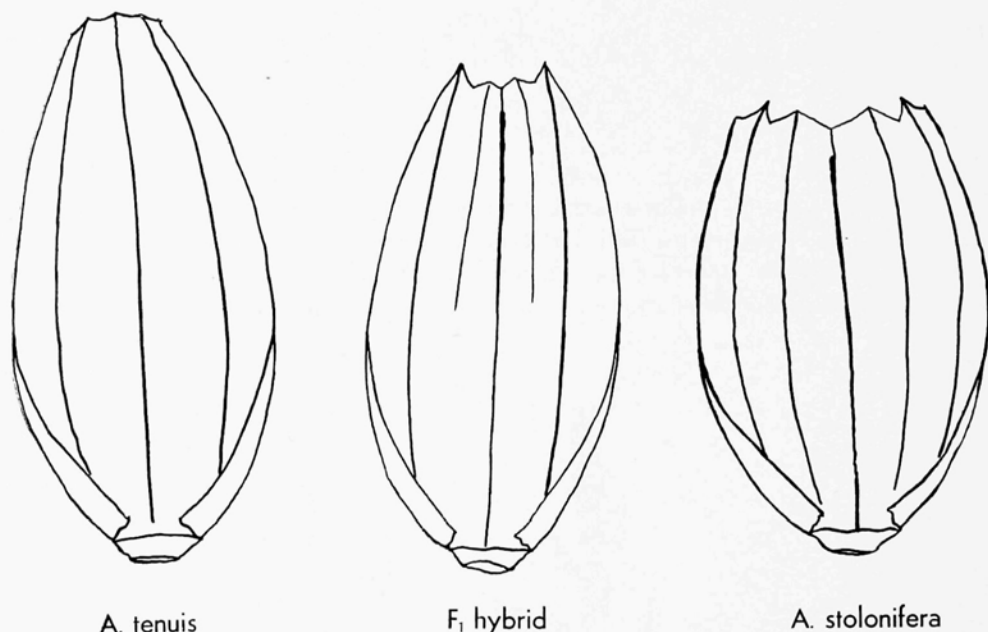


Fig. 3. Diagnostic characters of *Agrostis tenuis*, *A. stolonifera* and F_1 hybrid: lemmas.

the various characters in *Agrostis* this has not been done. No assumptions have been made as to which character is the most important, and all characters have contributed equally in the final index. The initial choice of characters was guided solely by those available and able to be measured without difficulty. Where only three different grades were distinguishable the scoring was as follows:

Resembling <i>A. tenuis</i>	0
Intermediate	2
Resembling <i>A. stolonifera</i>	4

Following the precept of Baker (1947), attention has been paid to the artificial hybrids in order to be certain that the index is effective with known hybrids. Fig. 5 gives the analysis of some plants from pure populations of *A. tenuis* and *A. stolonifera* and also the analysis of Davies's hybrids raised at the Welsh Plant Breeding Station.

The Oxford populations show the analysis given in Fig. 6. In the case of population B, which was grown at Aberystwyth, observations were only made on growth habit, ligule and panicle, and only three grades were observed. Thus a simplified index has been constructed.

Fig. 6 shows that the original speculations amply confirmed. Port Meadow contains a very large area (C) in which the *Agrostis* is almost exclusively intermediate between *A. tenuis* and *A. stolonifera* and is indistinguishable morphologically from artificial F_1 hybrids. Other areas contain predominantly *A. tenuis* (area A) and *A. stolonifera* (area D). Sample B, taken from various places in the meadow, shows a mixture of these three.

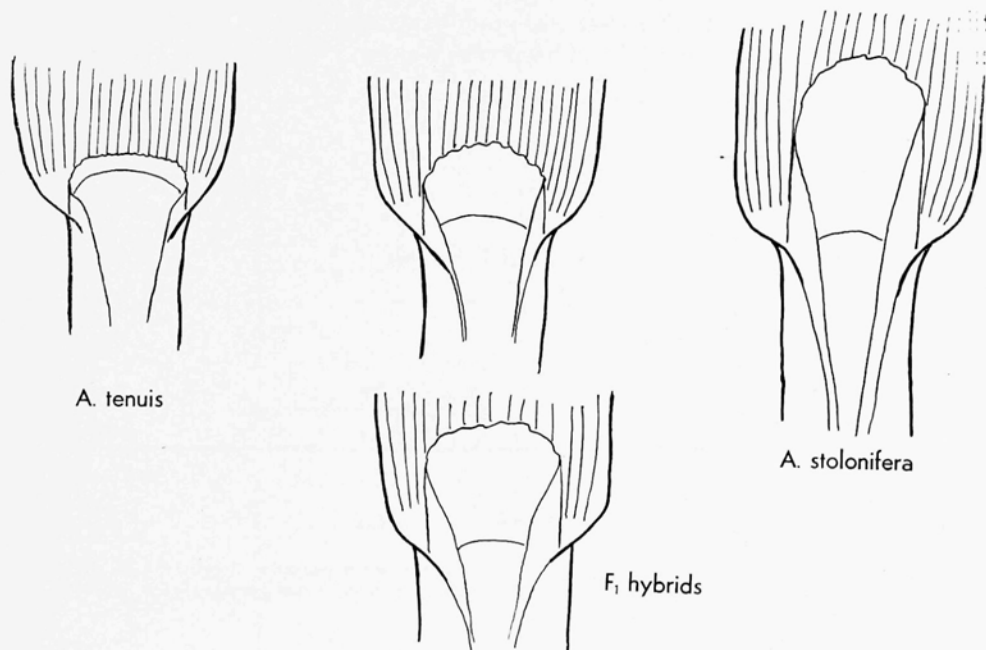


Fig. 4. Diagnostic characters of *Agrostis tenuis*, *A. stolonifera* and F_1 hybrid: ligules of vegetative shoots.

That this is so is confirmed by the fertilities of the plants. The pollen fertility was determined from material fixed in alcohol mounted in glycerine without staining. It was easy to determine the percentage of grains which were crumpled or devoid of dense contents. It was found essential to use only the absolute minimum of mounting liquid, otherwise, as the liquid flows to the edge of the coverslip, it tends to take with it the small crumpled grains which are not held between the coverslip and slide, and great apparent variations in fertility can be found on different parts of the slide. These estimations of pollen fertilities were followed up by measurements of seed fertility according to the amount of viable seed produced. But owing to insect damage and bad weather conditions at the time of flowering, only simplified results can be given. It can be seen in Fig. 7 that there is an excellent correlation between plant type and fertility. Plants of intermediate index values, supposed hybrids, have low fertilities with an almost identical range (0–37 per cent) and mean (13.1 per cent) to those of the synthetic hybrids given by Jones (1956). The same relationship can be shown for population B. There are, however, a few exceptions which will be discussed later.

STATUS OF INDIVIDUAL PLANTS

The obvious problem presented by these populations is whether they are made up of parents and F_1 hybrids only, or whether backcross and F_2 and other derivative types are

present. It is unfortunate that it has not yet been possible to produce artificial backcross and F_2 plants and to recognize the presence of these in the populations by direct comparison. But it is reasonably safe to assume that plants that seem neither to be like the parents, nor to conform with the known or expected characteristics of the F_1 types, must be backcrosses or other derivatives. Goodwin (1937), in analysing hybridization in *Solidago*, and Wetmore and Delisle (1939), in analysing hybridization in *Aster*, presumed that those plants, whose morphological index values came neither in the range of the parents nor the F_1 's were backcrosses and other derivative types. They showed that this presumption was born out by artificial backcross plants.

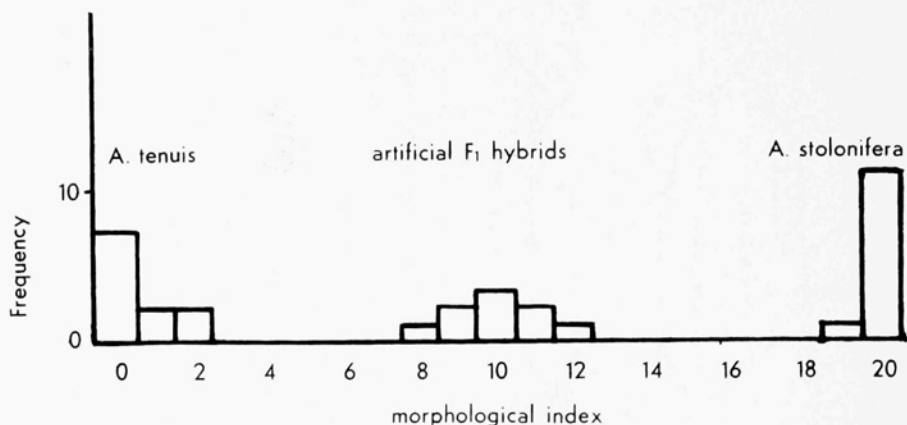


Fig. 5. Histogram for synthetic F_1 hybrid plants and their parents, together with other material of the pure species from Plynlymon, Cards. (*A. tenuis*) and Newborough Warren, Anglesey (*A. stolonifera*) (see p. 70).

This then is one approach, but it has the disadvantage that it is difficult to know what exactly are the ranges of variation of the parental and the F_1 plants, since experimental material need not behave exactly the same as the plants in the field. It is difficult to be certain that the plants being used in experimental crosses are a fair sample of the natural material. Jones (1956) in *Agrostis* and Stebbins (1945) more generally, have recorded that very different F_1 hybrids can be made when different plants of the same two species are used as parents of a particular cross. There is no simple way of overcoming this difficulty except by ensuring the experimental material is both sufficient and representative. Unfortunately, it was only possible to use for comparison Davies's hybrids which had been made from material collected mainly in Wales. But since the suspected natural hybrids agree in all respects very closely with the artificial ones, there seems no need for concern.

A second difficulty is that, although the method may work tolerably well for determining backcross plants, it will be of very little use for distinguishing F_2 plants. In these plants although recombination will be found, the individual characters, when combined into an index, will give values usually within the range of the F_1 . This is well shown by the histograms of the index values of F_1 and F_2 plants of *Solidago* given by Goodwin, where over 70 per cent of the F_2 plants fall within the range of the F_1 s. The key to recognition of these F_2 plants must lie in the recombination shown by the characters. For this reason, plants with intermediate index values which showed widely fluctuating character scores were classed as F_2 rather than F_1 types.

A third difficulty is that it is possible that backcross plants will fall within the range of the parents and so be indistinguishable from them. This is a further source of inaccuracy

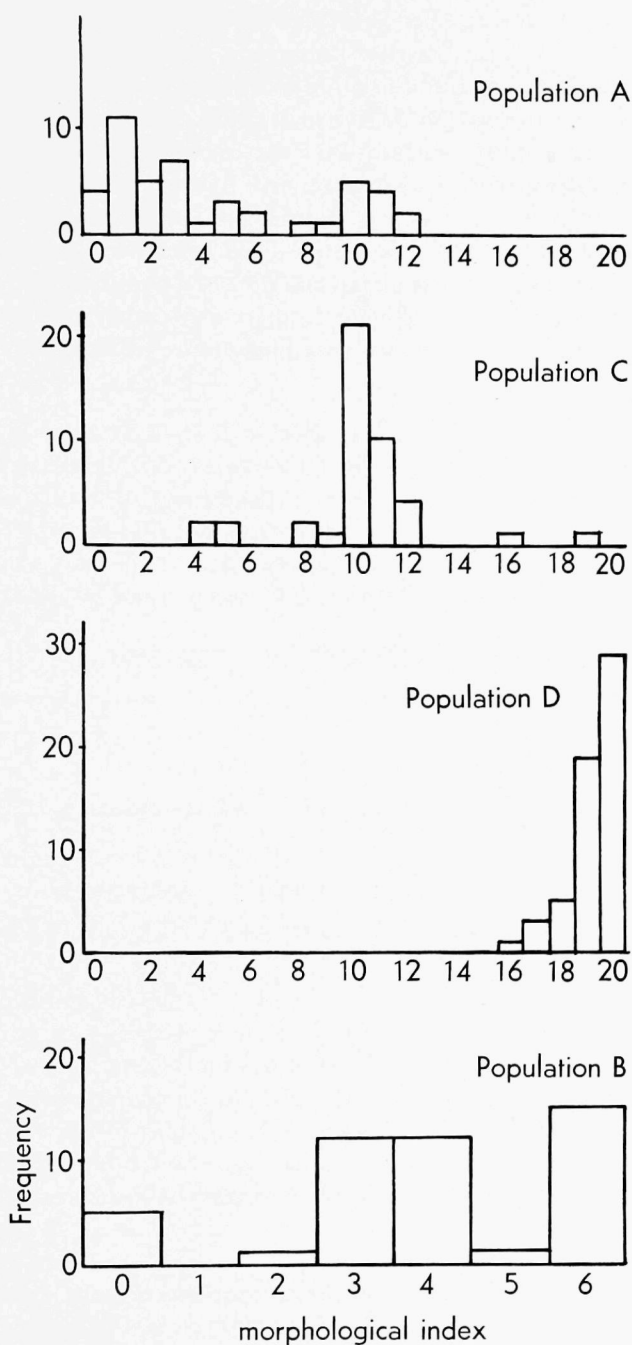


Fig. 6. Histograms of population samples of *Agrostis* (see text p. 71).

which may be considerable if the parental species are variable, and it may be impossible as a result to use the index method to analyse cases of introgressive hybridization when

the introgression is fairly complete. Anderson and Hubricht (1938) were unable to show introgression of *Tradescantia canaliculata* into *T. subaspera* by an index method of analysis, because the ranges of the two species were not sufficiently distinct, although from various kinds of evidence they suspected strong introgression. When, however, sterility is associated with hybridization, there is a possible way of recognizing backcrosses even when these are indistinguishable morphologically from the parents. Where F_2 hybrid plants show a reasonable degree of sterility, it is rarely found that backcross plants regain complete fertility, whatever be the cause of the sterility. The extensive work by Jenkin on interspecific and intergeneric hybrids in herbage grasses provides many examples to substantiate this, e.g. hybrids of *Lolium perenne* with other *Lolium* species (Jenkin, 1954). The backcross plants often resembled the parent in question closely. This therefore suggests that plants taken from a population where hybridization is occurring, which are indistinguishable from one or other of the parental species and yet are sterile or partially sterile, are very likely to be backcrosses of some sort.

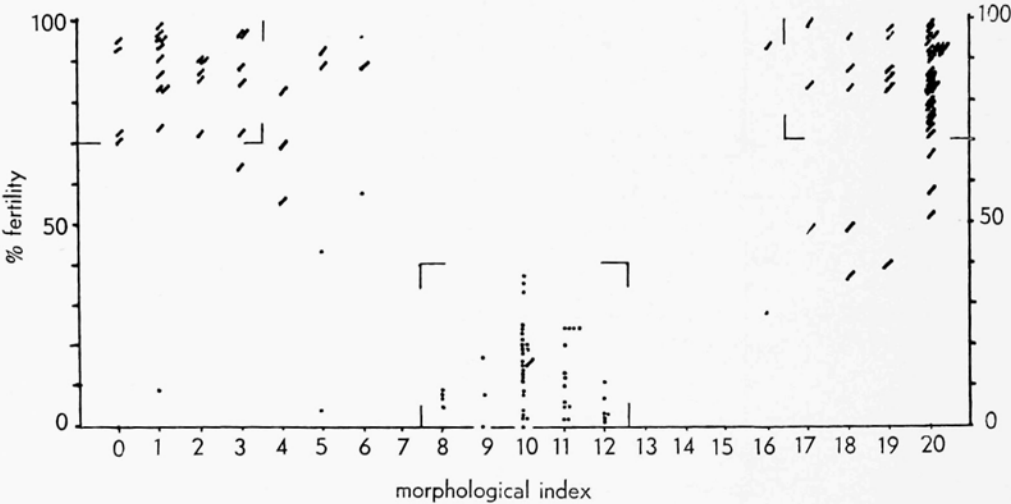


Fig. 7. Scatter diagram of fertilities of all plants of population samples A, C and D in relation to morphological index. The bracket lines indicate probable limits of the species and F_1 hybrid. • Little or no seed setting; | reasonable seed setting.

In the light of all this an attempt has been made to assess the status of each individual plant of the populations A, C and D on the basis of four categories, parents, F_1 hybrids, and derivatives (F_2 s and backcrosses), in order to obtain some idea of the relative proportions of the various types, especially the proportion of derivatives. The criteria of these categories are given in Table 2. Natural populations of the two parental species, and Davies's various artificial F_1 hybrids can be shown to fall into the first three categories very satisfactorily. It has not been possible to test the fourth category with artificial material.

Table 2. Criteria used to identify the various types of *Agrostis*

	Index value	Fertility	
<i>A. tenuis</i>	0-3	>70%	
<i>A. stolonifera</i>	17-20	>70%	
F_1 hybrids	8-12	>40%	Excluding those whose index value is made up of very fluctuating character scores
Derivatives	All those not included above		

Examples of individual plants of the various types are given in Table 3. The types are also indicated by the groups shown in Fig. 7. The plants considered to be derivative are those lying outside the marked groups, together with a few plants which are included in the intermediate group but which have very fluctuating individual character scores.

Table 3. *Examples of the different types of plant*

	Plant number	Growth habit	Ligule	Panicle	Pedice!el	Lemma	Index total	Fertility %
<i>A. tenuis</i>	A/16	0	1	0	0	0	1	73
	A/38	2	1	0	0	0	3	84
<i>A. stolonifera</i>	D/1	4	4	4	4	4	20	90
	D/31	4	4	4	4	2	18	87
F ₁ hybrid	C/2	2	2	2	2	2	10	15
	C/50	2	2	2	3	2	11	13
	C/41	2	2	1	2	2	9	17
Derivative	C/45	0	2	1	0	2	5	92
	A/2	3	2	0	2	3	10	8
	A/25	0	1	0	0	2	3	64
	D/3	4	2	4	3	4	17	48

This method of assessment is obviously not perfect, and in some cases inaccurate classification can occur. Plants belonging to the parental species, but sterile because of chromosomal structural heterozygosity or other causes entirely unconnected with hybridization, will be classified as derivatives. But these cannot be frequent and will only mean that the values for derivatives may be slightly overestimated.

Using this method the composition of the populations is that given in Table 4.

Table 4. *Percentage composition of populations on analysis*

Population	<i>A. tenuis</i>	<i>A. stolonifera</i>	F ₁ hybrids	Derivatives	No. of plants examined
A	52	0	31	17	48
B	11	35	54	not analysed	46
C			84	16	44
D		84		16	44

The status of a number of plants has been taken one stage further by Mr. Keith Jones, who has examined their cytology. Meiotic irregularity, of a type similar to that shown by the synthetic hybrids (Jones, 1956), was recognized by either the presence of univalents in pollen mother cell meiosis or micronuclei in the pollen tetrads. The results are given in Table 5 together with the status (from analysis) of each plant. There is a very

Table 5. *Meiotic regularity of plants in relation to their status determined by analysis (data by K. Jones)*

Population	<i>A. tenuis</i>	<i>A. stolonifera</i>	F ₁ hybrid	Derivative	No. of plants examined
A	6 regular		7 irregular	1 regular 5 irregular	19
B			4 irregular		4
C			13 irregular	2 regular 2 irregular	17
D		17 regular		2 regular	19

satisfactory correlation of meiotic upset with presumed hybridity. The only anomalies are in the case of some of the presumed backcross or derivative plants, sometimes these appear to possess meiotic abnormalities and sometimes they do not. Further analysis of them is necessary, but a variable behaviour is to be expected in such a category of plants. Chromosome counts were made on nearly all plants examined. They were all $2n = 28$

with one exception, which was $2n = 40 \pm$. It is likely that this has arisen by the fusion of an unreduced diploid gamete from a hybrid plant with a normal haploid gamete from a parental plant. The analysed status of this plant is 'derivative' which therefore agrees satisfactorily.

CHARACTERISTICS OF THE DIFFERENT TYPES

Its abundance shows that F_1 hybrid between *A. tenuis* and *A. stolonifera* is a plant that can compete very successfully with its parents and with other plants in certain situations. It is infrequent in very dry situations in Port Meadow and is not found at all in very wet regions. But in the large intermediate region 'C', it is extremely common, and appears to replace its parents in the sward. This could be due to the fact that it can grow in a habitat in which its parents are unable to grow. But this is not so, since both *A. tenuis* and *stolonifera* can be found growing in the habitat of the hybrid. Moreover in other regions known to the author *A. tenuis* and *A. stolonifera* are to be found growing together. Their ecological ranges overlap. This suggests that in Port Meadow the hybrid has in fact ousted the parental types from the sward. Quite apart from this it is clear that the hybrid possesses excellent competitive powers in relation to other grasses and can occupy up to 50 per cent of the sward in the middle of the Meadow. For this reason comparative studies have been made on the vegetative growth of the two species and the hybrid.

These analyses are on two lots of material. Firstly, the original population samples were grown as single spaced plants for three years in the experimental gardens at Bangor. When these populations were planted out, they were broken up into plots arranged in randomized blocks with six replications. The analysis of these plants was therefore as follows. The *A. stolonifera* plants which were measured were those in the population D plots in each block, the *A. tenuis* plants in the population A plots, and the hybrids in the population C plots. The analyses of variance were carried out on the means of the five to ten plants in each plot. Secondly, five plants of each type were grown in large pots in John Innes compost in an unheated greenhouse from February to October 1956. These greenhouse plants were all replicated clonally four times and the analysis has been carried out on the means of the five plants on each type. It was unavoidable that the plants representing the three types, i.e. *A. stolonifera*, *A. tenuis* and F_1 hybrid were taken from different populations, since no one population contained sufficient of all three. This might lead to the confusion of ecotypic differences with the differences in genetic type, since it has been shown (Bradshaw, 1954) that population differences can occur over relatively short distances. But in Port Meadow, although there is a definite edaphic gradient, the same general type of herbage (closely grazed dense turf) is found in all areas sampled. The grazing factor is relatively even throughout. So it is unlikely that any ecotypic differentiation of a morphological nature is occurring.

The analysis is based on single spaced plants. It has not been possible to compare the growth of the plants under grazing conditions. This is always a difficult problem in grasses, which are adapted to growth under grazing. It is not possible to judge directly the adaptation of the plant in its natural environment. But Stapledon and his co-workers have shown that certain characters such as spread, tillering, etc., which are of adaptive importance in grasses, can be observed from single spaced plants without erroneous conclusions about their value.

Table 6 shows that in general morphology the three types are quite distinct and that the F_1 hybrid possesses characters that adapt it very well to successful growth in a grazed sward.

Table 6. *Growth characteristics of parental and natural hybrid plants from Port Meadow*

	<i>A. tenuis</i>	F ₁ hybrid	<i>A. stolonifera</i>	Level of significance	Standard error of difference between means
SPREAD (cm.)					
in garden	18.3	23.8	20.6	.05	1.788
in greenhouse	80.0	117.25	116.5	.05	9.54
TILLER DENSITY (per sq. dm.)					
in garden	50.2	56.3	32.4	.001	5.86
in greenhouse	60.0	54.0	28.5	.001	2.1
HEIGHT OF VEGETATIVE GROWTH (cm.)					
in garden	16.78	12.68	11.0	.001	.74
DRY WEIGHT (gm.)					
in greenhouse	28.25	50.95	52.15	.01	5.79

A. stolonifera is a widely spreading diffuse plant of low height. It is a plant that survives in a sward by widely spreading stolons which send up tillers wherever there are spaces left by other plants. It rarely forms a dense continuous cover. Its growth habit is in many ways similar to that of *Trifolium repens*.

A. tenuis, however, is a rather tufted, dense plant considerably taller than *A. stolonifera*. By its tall dense growth it is able to compete directly with other plants, and indeed in rough grazings often forms over 50 per cent of the sward (Stapledon, 1936).

The hybrid seems to combine the ability to spread of *A. stolonifera* with the high density of tillers of *A. tenuis*. In fact it may even surpass either in these two characters. At the same time it is fairly low growing, considerably lower than *A. tenuis*. It therefore possesses exactly those characters which would adapt it to growth in a heavily grazed sward better than its parents. Its vigour, measured by dry weight, appears to be no better than that of *A. stolonifera* although considerably better than that of *A. tenuis*. It cannot therefore strictly be said to show heterosis, and its success cannot be due to superior vigour. There seems little difference between growth under garden conditions and under greenhouse conditions, probably because these two environments were not sufficiently distinct. But it is interesting that, although figures are not available, the superiority of vigour of the hybrid and *A. stolonifera* was not so marked under garden conditions, where fertility was lower and conditions were drier. Though further work is necessary, this presumably is related to the greater predominance of *A. tenuis* in population A. The hybrid has particular habitat requirements, those of a moderately damp fertile meadow, and is not able to compete so satisfactorily in the dry meadow.

An experiment providing a direct comparison of all these results with the artificial material of Davies at the Welsh Plant Breeding Station has not been possible. But Table 7 gives some observations of this stock material growing in pots outside. Under these conditions the growth is rather poor but the same general morphological relationships between the three types can be seen.

Table 7. *Growth characteristics of parental and artificial hybrid plants grown in pots outside (material of W. Ellis Davies)*

	<i>A. tenuis</i>	F ₁ hybrid	<i>A. stolonifera</i>	Level of significance	Standard error of difference between means
Spread (cm.)	8.13	17.36	20.78	0.001	2.20
Density (per sq. dm.)	70.0	68.3	38.3	0.001	6.9

The hybrid has strong powers of vegetative growth, predominates completely in the middle region of the meadow and is more or less sterile. It may well be asked whether the hybrid population is, in fact, due to the spread of one or only a few plants throughout the area, and is therefore composed of one or only a few clones. Port Meadow has been left as grassland so long that if a plant only grew at the rate of 50 cm. a year it could spread from the middle of the Meadow to all corners. This sort of situation has recently been suspected elsewhere (Harberd, 1958). On the other hand, however, Davies (1953) has shown that the hybrid can be produced with considerable ease and this would suggest that if the two parents grow together in the same sward new hybrids will be produced continually.



Fig. 8. Different types of panicle in natural F_1 hybrids of *Agrostis tenuis* and *A. stolonifera*.

On even a superficial inspection of population C, it is clear that several different types of hybrid are present. Fig. 8 gives some examples of the range of different panicle types that can be found. It must be made clear that the panicles produced by one plant are extremely consistent in shape, no matter whether they are produced from the middle or the edge of the plant and early or late in the season. It is therefore very easy to recognize differences of panicle shape between plants. At the same time there are very conspicuous differences in growth habit between different plants, differences of a sort which, while they can be recognized by eye, defy any analytical treatment.

An attempt has been made to recognize the different clones present in some of the populations. In the first instance plants were sorted in the laboratory into groups of apparently identical plants by means of collected panicles. These groups were then examined very carefully in the field to see firstly, whether members of each group really were similar in panicle shape and secondly, if so, whether they were also similar in vegetative growth. This analysis proved in fact to be easier than the author expected, and was eventually carried out on all the plants in populations A and C. *A. stolonifera* in

population D did not lend itself to this treatment, however, since the closed panicles made it impossible to distinguish different types and the vegetative growth, while showing definite differences between plants, was not sufficient for critical assessment. The results of the analysis are given in Table 8.

Table 8. *Cases of clonal reduplication in populations A and C (clones indicated by brackets)*

Plant type	POPULATION A Plant No.	Pollen fertility	Plant type	POPULATION C Plant No.	Pollen fertility
<i>A. tenuis</i>	{ A/40 A/59	85 90	<i>F</i> ₁ hybrids	{ C/6 C/12 C/18 C/55	20 19 20 24
<i>F</i> ₁ hybrids	{ A/5 A/7 A/17	2 5 2		{ C/25 C/30	8 9
	{ A/2 A/46	8 9		{ C/9 C/14	24 25
	{ A/41 A/48	12 12		{ C/41 C/46	17 15
Derivatives	none			{ C/53 C/54	22 20
			Derivatives	none	

Although not used in the analysis, attention was paid to the pollen fertilities of the plants in the groups. After the first separation on the basis of panicles, the groups often contained plants with widely different pollen fertilities. But after the final separation in the field, in no case did any group possess plants with fertilities differing by more than 5 per cent. The fertilities are given in Table 8, and are excellent confirmation that the plants in each group are members of one clone.

The amount of duplication is therefore very small, especially in relation to the size of sampling areas which were 50 m. in diameter. It suggests that the hybrid must have been formed anew on countless occasions. The duplication is not sufficient to affect the earlier analysis and therefore has been disregarded in the earlier part of this paper.

It is possible from this analysis, and from the relative size of the sampling area, to get some idea of the number of hybrid plants in the meadow. Since the sampling area cannot be more than 1/500th of the total area of the meadow occupied by the hybrid, it seems likely that the number of different *F*₁ hybrid plants must be in the neighbourhood of 20,000. This is considerably more than that recorded for *Elymus glaucus* × *Sitanion hystrix* (Stebbins, 1952). It most certainly correlates with the known ease of production of the hybrid (Davies, 1953).

INCIDENCE OF 'CHOKE' (*EPICHLÖE TYPHINA*)

In all populations sampled about one-eighth of the plants were found to be infected with the fungal parasite 'Choke', *Epichloe typhina*. This prevents the plant from producing panicles without in any other way weakening the plant. In fact there is evidence that the presence of 'choke' has a favourable effect on the vegetative growth of the plant, increasing the density of vegetative tillers (Bradshaw, unpubl.). Because of the absence of panicles and the possibility of modified growth, these plants have been left out of all the preceding analyses. Analysis of the plants on vegetative characters show that the affected

plants are of all types. There seems to be little difference in susceptibility of the different species or the hybrid, for in each population, the particular frequencies of each type follow the general composition of the population concerned. Because of this the removal of these affected plants from analyses causes no serious bias. But since these plants do constitute quite a significant proportion of the population, they must be considered as an element of it.

OCCURRENCE OF SIMILAR POPULATIONS ELSEWHERE

Similar populations have so far been discovered in the following localities:

Permanent pasture on heavy clay near Waterperry Wood, Oxfordshire; permanent pasture on valley alluvium near Aberystwyth, Cardiganshire; damp stabilized sand dune grassland on Newborough Warren, Anglesey; hay meadow on damp alluvium at Pixey Mead, near Oxford.

The most extensive amounts of *A. tenuis* \times *stolonifera* were in the first two of these. From preliminary observations and from the known ease of production of the hybrid, it seems probable that hybrid populations are quite common in conditions (a) of permanent grazed grassland and (b) where conditions suit both parents, i.e. in rather damp neutral or slightly acidic grassland. Such grasslands are very common in the British Isles, and the presence of the hybrid within them may go far to explain why competent experimental workers have so often been forced to record *Agrostis* merely as *Agrostis* sp. Many other areas are known to the author where undisputedly hybrid plants are to be found, but so far no information is available as to their frequency.

DISCUSSION

Ecology

The most unusual feature of the hybridization of *A. tenuis* and *A. stolonifera* is clearly the remarkable frequency of the F_1 hybrid in the face of heavy competition. Hybridization between plants usually depends, amongst other things, on the availability of open ground in which the hybrids can develop and it is for this reason that cases of hybridization are so often associated with disturbed and open habitats, e.g. *Salvia apiana* \times *S. mellifera* (Anderson and Anderson, 1954). In this case of *Agrostis*, however, the hybrids have become more frequent than the parents in a closed community. The grassland of Port Meadow is likely to have been in existence long before its recorded mention in the Domesday Book, and must be considered as a closed community in relation to invasion by grasses. *Agrostis tenuis* \times *stolonifera* has a growth habit well adapted to a grazed sward, and must have been able to compete more than equally with the other grasses and with its parents.

Many other natural sterile F_1 hybrids have good powers of growth. But most of these lack the power of vegetative reproduction, spread and therefore competition possessed by *Agrostis*. A notable exception is *Vaccinium* \times *intermedium* (Ritchie, 1955a, 1955b), which seems to possess even stronger powers of growth than its parents, and is able to spread into areas already occupied by them. The *Gramineae*, because of their powers of vegetative reproduction, provide several other instances. Thus \times *Festulolium loliaceum*, the hybrid between *Festuca pratensis* and *Lolium perenne*, has great vigour and powers of persistence (Jenkin, 1933). It is found in quantity in Port Meadow. A similar case is the hybrids between *Stipa cernua*, *S. pulchra* and *S. lepida* which are common in parts of

America and which have been shown experimentally to be vegetatively vigorous (Love, 1946). *Glyceria* \times *pedicellata*, from *G. fluitans* and *G. plicata* is very common in many slow moving streams. *Alopecurus* \times *hybridus* from *A. geniculatus* and *A. pratensis* is often to be found in abundance with its parents.

These and others are cases of extensive areas of natural F_1 hybrid grasses in Britain which would be worth examination. But many of them such as \times *Agropogon littoralis*, *Agropyron junceiforme* \times *pungens*, and perhaps *Glyceria* \times *pedicellata*, are not really in the same category as *Agrostis tenuis* \times *stolonifera* because they are only to be found in open habitats where there is little competition. \times *Agropogon littoralis* for instance usually only occurs in open habitats and has definitely weaker powers of vegetative growth than its vegetatively vigorous parent, *Agrostis stolonifera*. Nevertheless these are all interesting plants.

Another aspect of the success of naturally occurring sterile hybrids is that they are often able to occupy habitats different from those of their parents, thereby escaping at least the direct competition of their parents even if not the competition of other plants. There is perhaps some indication that part of the success of *A. tenuis* \times *stolonifera* is due to this. Although *A. tenuis* and *A. stolonifera* will grow in the habitat of the hybrid, it is quite clear that such habitats are not their most typical. *A. tenuis* is most common in drier areas poor in bases, and *A. stolonifera* is most common in wetter areas. In the case of \times *Festulolium loliaceum* preliminary observations suggest that the position is similar. The hybrid, by its growth habit, is adapted to moderately grazed pastures, while *Lolium perenne* is adapted to heavily grazed pastures, and *Festuca pratensis* to pastures where grazing is very light. One element of the success of *Vaccinium* \times *intermedium* seems to be its ability to spread into areas which its parents are unable to colonize. (Ritchie, 1955a). A very good case of this is provided by *Vaccinium elliotii* \times *tenellum* in grazed and burnt valleys of Georgia (Darrow and Camp, 1945). The grass excludes the low growing *V. tenellum* and burning excludes the tall *V. elliotii*. The hybrid persists by a combination of underground rhizomes and vigorous high growth.

In many of the above cases it is clear that Anderson's concept of hybridization of the habitat (Anderson, 1948) applies, as one might expect, to these F_1 hybrids. The hybrid is most suited to a habitat intermediate to those of its parents. A series of examples of this in the *Hordeae* is given by Stebbins, Valencia and Valencia (1946). As far as edaphic conditions are concerned, *A. tenuis* \times *stolonifera* seems to be strictly intermediate in its requirements. But there are a few cases where the optimum habitat of the hybrid is not intermediate to those of its parents. *Elymus condensatus* \times *triticoides* (Stebbins and Walters, 1949) occupies the edges of fields, roadsides, railways, etc., throughout a large area of California, habitats which are not strictly intermediate between those of its parents. *Vaccinium elliotii* \times *tenellum* reaches into valleys well away from the upper reaches and head-waters where the two parental species come together. There is an element of this type of behaviour in *Agrostis tenuis* \times *stolonifera*. It is better adapted to pasture conditions than either parent, in this character showing a transgressive rather than an intermediate behaviour.

Genetics

It is true that there has been difficulty in the past in differentiating *Agrostis tenuis* from *A. stolonifera* but it is quite clear that the two species are really very distinct in the British Isles. Most difficulties come in attempts to identify the two species solely on their vegetative characters, and arise from the fact that the characters available are very

much modifiable by environmental conditions, especially grazing and dryness. Thus although the F_1 hybrid is easily produced, there seems to be no tendency for the merging of the two species.

While the distributions of the two species do overlap considerably, there are vast areas where the two species are separate. *A. tenuis* is commonly found on base poor soils both dry and wet of pH 4-6.5. *A. stolonifera* is restricted to base rich soils of pH 6 and upwards. There is, therefore, a strong measure of ecological isolation. On the other hand there are numerous lowland areas where the two species do meet.

The isolation must be aided considerably by the sterility of the F_1 hybrid. Pollen fertilities of 20 per cent imply a much lower actual capacity to produce successful offspring, perhaps 5 per cent or less. Since it seems unlikely that the fertility is restored immediately on backcrossing, the possibility of gene flow from one species to the other is likely to be considerably below even this figure, since the barrier of sterility, if it affects several generations, will act in a geometric fashion. This is borne out by the very few definite backcross plants.

But in hybridization between *Helianthus annuus* and *H. bolanderi* similar sterilities seem to have been no real barrier to gene flow between the two species (Heiser, 1949). In this case, however, the hybridization has been associated with considerable habitat disturbance. This will have caused large areas of new open habitats in which all hybrid derivatives would have been able to grow. This is not perhaps the case with *Agrostis tenuis* \times *stolonifera*. Another point is that the sunflowers are annual, while *Agrostis* is perennial and strongly vegetative. Baker (1951) suggests that this may interfere by crowding with the development of seedlings. In *Agrostis* it certainly does not prevent the establishment of the F_1 . Whether the vigour of the F_1 prevents the establishment of subsequent generations is another matter. But few communities are completely closed and there would always be opportunities for these plants to get established if they were vigorous enough.

However, until further work is carried out, the amount of gene flow between *A. tenuis* and *A. stolonifera* cannot be determined. A comparison of the natural populations of Fig. 5 with the Port Meadow populations of Fig. 6 suggests that there may be some gene flow affecting both species in Port Meadow. In the Port Meadow populations the frequency distribution diagrams of the two species do show a spread in the direction of each other not present in the populations shown in Fig. 5. An examination of habitats containing the two species may well yield some populations with considerable amounts of introgression.

General

Agrostis tenuis \times *stolonifera* is a species hybrid of the pauciform type (Allan, 1937). It does, however, show some diversity and must be of multiple origin. The same situation has recently been reported in *Glyceria* \times *pedicellata* (Borrill, 1956). That a plant such as *G.* \times *pedicellata*, as well as *A. tenuis* \times *stolonifera*, does show diversity is interesting since although the latter has only limited powers of dispersal the former is able to be dispersed over long distances by water. It suggests that multiple origin of sterile F_1 hybrids is usual. If the hybrid can be formed once it is reasonable to assume that it can be reformed again elsewhere. Since nearly all species have a considerable amount of ecogeographical differentiation this must mean that we are likely to find different forms of the same hybrid occurring in different regions. These forms will be related to the forms of the parental species. The magnitude of these differences is likely to be much greater than the

differences described between individual plants of the Port Meadow populations. It may be sufficient to cause confusion and difficulty in the recognition of the hybrid.

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