SELECTION AND POPULATION STRUCTURE IN *LOLIUM*

III. SELECTION FOR DATE OF EAR EMERGENCE

J. P. COOPER

Welsh Plant Breeding Station, Aberystwyth

Received 7.ii.59

1. INTRODUCTION

IN a previous paper (Cooper, 1959*b*) it was shown that in both Irish and Kent ryegrass most of the variation in date of ear emergence was genetic and additive, and that all the plants tested were highly heterozygous for genes controlling this character. Considerable response to selection should therefore be possible. The present paper describes the actual response within these two populations during the first three generations of selection for early and late heading. The experiment was designed to compare the effects of different mating systems and initial population sizes on the response to selection, and also to assess the amount of potential genetic variation carried within one plant or a small group of plants. In addition, during summer 1957, ear measurements were made on the initial populations, and on parents and progenies of all three generations to detect any correlated response to selection, and to assess the effect of different degrees of inbreeding on these characters. These results will be discussed in a later paper.

2. MATERIAL AND METHODS

The two initial populations of Irish and Kent ryegrass consisted of commercial samples from the same merchant. In each year, beginning in 1953, seedlings and clonal material of the parents of all generations were planted out in the autumn, and the date of ear emergence was recorded the following spring. Details of the sowing and planting dates have been given earlier (Cooper, 1959*b*). The plants to be used as parents in each year were lifted immediately after ear emergence, potted and removed to the greenhouse for crossing. In this way a generation time of one year was obtained. The initial selection in summer 1954 was made in the Agricultural Botanic Garden, University of Reading, but all subsequent generations were tested at the Welsh Plant Breeding Station, Aberystwyth.

(i) *Mating systems*

The first crosses were made in summer 1954. The earliest and latest four plants out of 125 were lifted from Irish and Kent ryegrass, each was selfed, and the plants within each group were crossed in pairs. These crosses were made without emasculation according to the mutual pollination method of Jenkin (1931). This may result in a small proportion of selfing but does not affect the present conclusions (Cooper, 1959*b*).

From these crosses, four main mating systems were established as shown in fig. 1, the aim being to derive early and late lines from each initial group, and so to assess the variation carried within one plant, within two, and within a group of four.

(a) *Four-plant lines (Fourway crosses)*. In the first generation only pair-crosses were made between the four plants of each group, but in the next year, 1955, the earliest and the latest two plants were selected from two complementary pair-crosses within each group; the four early plants were allowed to intercross and also the four late ones. This gave rise to four selection lines from each strain, an early/early, early/late, late/early and late/late, denoted EE, EL, LE and LL respectively. This mating system was continued in subsequent generations by intercrossing the four extreme plants, either early or late, within each line. Each pair of lines is thus based on four unrelated plants (8 different chromosome sets) from the same population.

(b) *Two-plant lines (Sib-crosses)*. In the second generation in 1955, the two complementary pair-crosses used to produce the fourway lines were each continued by sib-mating. The two earliest and two latest plants from each progeny were selected,

FIG. 1.—Mating systems used in selection experiment. (A, B, C, D represent the original parents of each selection group, while E₁, E₂ etc. and L₁, L₂ etc. represent the plants selected for earliness or lateness respectively in subsequent generations.)

and from these EE, EL, LE and LL lines were derived. Starting with two pair-crosses, this gave two lines of each kind from each strain. Each pair of lines is thus based on only two plants (4 different chromosome sets).

(c) *Single-plant lines (selfing followed by sib-mating)*. Of the 8 initial parents of each strain selfed in 1954, only 4 of Irish and 5 of Kent produced more than 20 seedlings. From these, two early and two late progenies were selected in each strain, and from each progeny an early and a late line were derived by sib-mating. These lines were continued by sib-mating in subsequent generations, giving two lines of each type for each strain. Each pair of lines is thus based on only one plant (2 different chromosome sets).

(d) *Single-plant lines (continued selfing)*. It was hoped to establish early and late lines from within the selfed progenies by continued selfing. Self-sterility and lack of vigour, however, made this impossible, although a few lines were maintained to the third generation.

In addition to these main mating systems, fourway crosses were made in 1956 between the two duplicate sib-mating lines and also between the two duplicate selfing/sib-mating lines within each selection group, to study the effect of relaxing inbreeding while maintaining the direction of selection.

The selection differentials and the coefficients of inbreeding for the different mating systems are given in table 1. The coefficients of inbreeding are calculated on the assumption that selfing does not occur in the fourway crosses or pair-crosses. The actual amount of selfing is small, and would result in only a slight increase in the coefficient of inbreeding. The range in the selection differentials for the single-plant lines is a result of the low survival of inbred material, the number of plants recorded in each progeny varying from 10 to 50. In the selfed lines, little effective selection was possible.

(ii) *Experimental layout*

In each year, seedlings from the original populations and from progenies of all generations were put out as spaced plants at 2 feet apart, together with clonal plants of the parents of all generations. Fifty seedlings of each progeny were used where possible. Smaller progenies, which occurred mainly in the single-plant lines, were not used unless 10 seedlings were available. In 1957 some 5000 seedlings and 1250 clonal plants were recorded.

TABLE 1

Coefficients of inbreeding and selection differentials under the mating systems employed

Mating system	Original population		First generation		Second generation		Third generation
	Coeff. of inbreeding	Selection differential	Coeff. of inbreeding	Selection differential	Coeff. of inbreeding	Selection differential	Coeff. of inbreeding
		Per cent.		Per cent.		Per cent.	
Fourway . . .	0.0	3.2	0.0	4.0	0.125	8.0	0.219
Sib-crosses . .	0.0	3.2	0.0	4.0	0.25	4.0	0.375
Sib-crosses/Fourway	0.0	3.2	0.0	4.0	0.25	4.0	0.25
Selfing/Sib-crosses	0.0	3.2	0.5	20.4	0.5	4.0	0.625
Selfing/Sib-crosses	0.0	3.2	0.5	20.4	0.5	4.0	0.35
/Fourway							
Selfed lines . .	0.0	3.2	0.5	20.2	0.75	20.2	0.875

The results from clonal replication given earlier (Cooper, 1959*b*) suggested that block differences would not be significant, and that genotype/block interaction would be rather less than within-plant variation (developmental error). Consequently, in the experimental layout, the *progeny* seedlings were not replicated, but the clonal parents and the *population* seedlings were replicated in five blocks. As shown in table 8, no significant block differences could be detected, nor were there differences in within-plant variation between generations or between mating systems. The clonal replicates could therefore be used to give a satisfactory estimate of within-genotype variation.

3. RESULTS: MEAN RESPONSE TO SELECTION

In studying the results of any selection programme, two main effects are of interest ; firstly, the mean response to selection in relation to the selection differential applied, and secondly, the changes in genetic variation which occur within and between the lines.

A summary of the response to selection under the different mating systems in 1957 is given in table 3, while the behaviour of the fourway lines, and certain of the sib-mating lines, is shown in figs. 2 and 3. The results for 1956 in which only two generations were recorded are similar to those for 1957 and are not presented here. The environmental and within-plant errors for each generation or mating system can be obtained from table 8.

TABLE 2

Dates of ear emergence 1954-57; original populations and selected parents

		Reading 1954	Aberystwyth 1955	Gogerddan 1956	Gogerddan 1957
Irish					
Original population	Mean Var. Range	3.4 May 24.0 23 Apr.- 14 May	14.0 May 30.7 4.25 May	8.7 May 12.4 1.17 May	30.4 Apr. 18.7 18 Apr.- 18 May
Early parents . . .	No. 1 2 3 4	23 Apr. 24 Apr. 24 Apr. 25 Apr.	28.5 Apr. 6.5 May 28.5 Apr. 2.5 May	1.2 May 8.2 May 2.8 May 5.4 May	24.6 Apr. 1.4 May 24.6 Apr. 30.5 Apr.
Late parents . . .	No. 1 2 3 4	14 May 13 May 14 May 11 May	26.5 May 24.5 May 24.0 May 19.5 May	21.4 May 19.6 May 16.0 May 14.6 May	16.0 May 13.2 May 6.8 May 9.2 May
Kent					
Original population	Mean Var. Range	24.1 May 26.4 9 May- 3 June	29.7 May 24.5 19 May- 12 June	22.5 May 18.0 11.30 May	22.8 May 40.0 3 May- 5 June
Early parents . . .	No. 1 2 3 4	13 May 9 May 11 May 13 May	22.0 May 17.0 May 15.0 May 17.0 May	18.4 May Dead 15.8 May 18.0 May	12.2 May Dead 10.8 May 12.0 May
Late parents . . .	No. 1 2 3 4	2 June 2 June 3 June 2 June	2.5 June 3.5 June 7.0 June 3.5 June	30.6 May 8.2 June Dead 31.2 May	29.6 May 3.4 June Dead 2.0 June

(i) *Initial material*

The heading dates for 1954-57 of the original populations and of the initial parents are shown in table 2. There is a regular difference of three to four weeks in mean heading date between Irish and Kent ryegrass, but the latest plants of Irish overlap the earliest of Kent.

TABLE 3
Response to selection 1957

	Irish				Kent			
	30.4 April 28.7 (18 A-18 M)				22.8 May 40.0 (3 M-5 J)			
<i>Original Population</i>								
Mean . . .								
Var. . . .								
Range . . .								
<i>Third generation lines</i>								
A. Fourway crosses								
Mean . . .	20.8 A	9.9 M	6.4 M	13.0 M	22.7 M	25.4 M	8.7 J	
Var. . . .	21.9	29.7	15.8	18.8	12.3	15.7	10.0	
Range . . .	(10-20 A)	(30 A-18 M)	(29 A-15 M)	(5-23 M)	(15-30 M)	(17 M-2 J)	(2-16 J)	
B. Sib-crosses/fourway								
Mean . . .	18.9 A	2.6 M	3.9 M	14.8 M	17.5 M	27.8 M	9.6 J	
Var. . . .	17.8	30.1	41.3	26.8	17.6	7.6	30.7	
Range . . .	(10-23 A)	(23 A-16 M)	(24 A-12 M)	(8-23 M)	(11-28 M)	(24 M-2 J)	(30 M-21 J)	
C. Selfing/sib-crosses/ fourway								
Mean . . .	17.7 A	...	1.8 M	11.1 M	...	...	...	
Var. . . .	15.1		15.9	23.3	9.1			
Range . . .	(10-23 A)		(27 A-13 M)	(4-25 M)	(20 A-3 M)			
D. Sib-crosses								
Mean . . .	19.2 A	30.0 A	2.3 M	17.0 M	24.9 M	18.5 M	...	
Var. . . .	38.6	24.5	12.1	37.2	40.8	20.7	28.4 M	
Range . . .	8-30 A	23 A-9 M	27 A-11 M	8-31 M	23 A-24 M	15 M-5 J	11-21 J	
E. Selfing once/sib-crosses								
Mean . . .	20.8 A	7.1 M	1.7 M	15.3 M	...	...	...	
Var. . . .	32.8	19.0	31.6	9.3	7.1	12.4	...	
Range . . .	10 A-4 M	17 A-4 M	24 A-16 M	12-25 M	20-31 M	13-27 M	...	
F. Continued selfing								
Mean . . .	...	6.5 M	29.6 A	...	...	...	...	
Var. . . .	...	14.5	16.3	...	...	...	...	
Range . . .	...	30 A-13 M	23 A-8 M	...	...	...	...	

... indicates that the line had died out by the third generation.

Kent usually shows a greater variance than Irish, and there is considerable variation between the early year 1957 and the late year 1955, this difference being greater for Irish than for Kent. A more

FIG. 2.—Response to selection for ear emergence in Irish and Kent ryegrass. Fourway crosses 1957.

— Mean of selection lines
 - - - - Extreme early and late plants

detailed discussion of the heading behaviour of these strains has been presented earlier (Cooper, 1959a).

The parents selected as the earliest and latest in 1954 at Reading remained early and late respectively in subsequent years at Aberystwyth.

They did not, however, always represent the extreme range of the original population, particularly in the early year 1957, when in Irish ryegrass the clonal material of the original early parents was

FIG. 3.—Response to selection for ear emergence in Irish and Kent ryegrass. Sib-crosses 1957.

— Mean of selection lines
 - - - - - Extreme early and late plants

some nine days later than the earliest of the population seedlings, and that of the late parents some six days earlier than the latest population seedlings.

(ii) First generation

The first generation of selection proved to be highly effective, as would be expected from the high additive genetic variation in the original populations (Cooper, 1959*b*). The progenies from selfing or pair-crossing, however, showed genetic variances of the same order as those of the original populations, indicating the heterozygous nature of the selected parents. The progenies from selfing showed marked inbreeding depression, being about five days later in both Irish and Kent, and this obscured the response towards earliness in the single-plant lines. As would be expected from the overlap of their parental distributions, the Irish late and Kent early groups were of similar heading date, and similar variance. The means of the early and late selection lines lay within the range of the original populations, but the extreme early and late plants were well outside. The same pattern of response was noted in all the three years, 1955-57, although in the cold spring of 1956, all dates of heading were later and the range between early and late material was reduced, compared to the warm spring of 1957.

(iii) Second and third generations

In these subsequent cycles of selection, the genetic variation available was that derived from a small group of plants of similar phenotype, and the response would not necessarily be as great as in the first generation. However, all the selected parents appear to be very heterozygous, and response continued during these two generations, although at a reduced rate in some lines. By the third generation, the extreme plants from both Irish and Kent were well outside the range of the original populations, and in Kent the *means* of the extreme EE and LL lines exceeded the original population range. Similarly, the late lines from Irish covered much of the range of the original Kent, and the early lines from Kent most of the range of the original Irish. (By the fourth generation, recorded in 1958, the late lines from Irish cover the full range of Kent ryegrass, and the early lines from Kent cover the full range of Irish.) There is no sign that the limits of selection have been reached, but this would not be expected by only the third generation. (The apparent lack of response in the LE and LL fourway lines in Irish does not continue in the fourth generation.) Evidently the genetic variation carried within even one individual is not yet exhausted.

The rate of response is not, however, the same for all groups and for all mating systems, and more detailed information on the efficacy of selection is presented in tables 4 and 5.

Table 4 shows the effect of each particular parental group on the response to selection, the response being measured by the difference between the means of the early and late lines from within each original parental group; only the fourway crosses and the sib-mating/fourway outcrosses are included in this table.

This divergence is greater in the lines derived from early than from late parents, and is also greater in Kent than in Irish. This is particularly noticeable when the response from within the four late Irish plants is compared with that from the four early Kent plants. These initial groups have roughly the same heading date, but the early Kent

TABLE 4

Influence of parental groups on mean response to selection. Difference between means in third generation, 1957

	Irish			Kent			Kent-Irish	
	E/L-E/E	L/L-L/E	Difference	E/L-E/E	L/L-L/E	Difference	Early	Late
Fourway	19.1	6.6	12.5 (± 1.31)	24.5	14.3	10.2 (± 1.12)	5.4 (± 1.34)	7.7 (± 1.10)
Sib-crosses/ Fourway	13.7	10.9	2.8 (± 1.52)	21.4	12.8	8.6 (± 1.17)	7.7 (± 1.25)	1.9 (± 1.46)

TABLE 5

Differential response to selection for early and late ear emergence 1957

	Early/early lines			Early/late lines			Late/early lines			Late/late lines		
	Selection Differential (days)		Response (days)	Selection Differential (days)		Response (days)	Selection Differential (days)		Response (days)	Selection Differential (days)		Response (days)
	a	b		a	b		a	b		a	b	
<i>Irish (family means)</i>												
Fourway crosses . . .	1.6	14.8	3.5	18.0	17.8	13.0	2.0	10.9	3.0	19.7	8.0	5.8
Sib-crosses . . .	1.1	16.6	2.2	16.7	16.1	11.2	3.0	10.4	4.5	21.0	7.9	9.9
<i>Kent (family means)</i>												
Fourway crosses . . .	5.1	13.8	8.7	22.0	10.9	14.2	-0.5	10.9	5.0	16.0	18.2	9.8
Sib-crosses . . .	7.0	14.8	5.6	20.5	8.5	15.8	-3.3	14.1	2.0	22.2	12.8	18.1

a = calculated from clones and progenies in 1957.

b = calculated from parental records in 1955 and 1956.

group gave more than twice the response than did the late Irish. Two reasons for this difference may be suggested. Firstly, Kent ryegrass has been less stringently-selected for uniformity of ear emergence than has Irish (Cooper, 1959a). Secondly, while late heads of an early-flowering genotype might be included in the seed crop of the late-flowering Kent, no such introgression is possible in an early-flowering strain.

More detailed information is provided in table 5 in which the responses to selection in the two directions were recorded separately and measured as the difference between the means of the first and third generation progenies. The cumulative selection differentials for these two generations have been expressed in two ways: (i) as the actual divergence in 1955 and 1956 between the family means and the parents selected from them, and (ii) as the difference between these same family means and clonal material of the selected parents when all were recorded in 1957. In this way it was hoped to assess the effect of genotype/year interaction on the selection differential.

Three important points arise from this table: (a) the response to selection is asymmetrical in all groups, the advance towards lateness being greater than that towards earliness, although the original selection differential was of the same order in both directions, (b) the response is of the same order in all the mating systems mentioned, there being no regular difference between the lines derived from four plants or from two plants, (c) there is a striking contrast between the *actual* cumulative selection differentials applied in 1955 and 1956, and the differences observed in 1957 between the corresponding family means and the clonal material of the parents selected from them. This is particularly noticeable in the lines selected for earliness, whether from early or late parental groups. In these lines, both the selection differentials and the observed responses as recorded in 1957 are comparatively small, but the cumulative selection differentials calculated from 1955 and 1956 data are far higher. The realised heritabilities therefore are high when calculated from the 1957 records but low when based on the actual selection differentials applied during the experiment. In the late lines, the agreement between the two measures of selection differential is better and the realised heritabilities are closer to the expected figures of 0.7-0.9 derived from parent/progeny regression within a single year (Cooper, 1959*b*).

These differences in selection differential from year to year may be due to genotype/year interaction. Selection for early heading can involve either a lower critical photoperiod, or the ability to grow at lower temperatures, and the particular mechanism selected may depend on the spring temperatures in the year of selection. It may be relevant to the present results that 1957 was a very warm spring, whereas in 1955 and 1956 the spring was cold. (Both the asymmetry in response, and the greater release of variation from Kent than from Irish, are confirmed in the fourth generation of selection in 1958.)

(iv) *Effect of mating system*

Up to the third generation, there has been no regular difference in the extent of response between the lines derived from one, two or four parents. The genetic variance within the lines derived from even one individual remains high, and the potential genetic variation within the initial groups is far from exhausted. Outcrossing in the

third generation (table 3) did not greatly influence the extent of response although in most cases the mean of the outcrossed sib-lines was earlier than that of the corresponding sib-crosses. Only enough single plant lines survived to produce four of the eight potential outcrosses, but here again the outcross means were all earlier than those of the corresponding sib-crosses. This increase in earliness may be a result of relaxation of inbreeding.

The most obvious effects of the different mating systems have been those associated with survival. In the extreme case of continued selfing (table 3) only five lines of Irish and five of Kent survived to 1956, and only three of each strain to 1957. Even in the initial selfings followed by sib-mating, only nine out of the sixteen potential lines could be maintained to 1957; in the sib-mating three lines out of sixteen have disappeared. This failure to maintain the inbred material is due partly to self-sterility and partly to low vigour and poor survival of the seedlings.

TABLE 6
Release of variation from initial groups (range in days), 1957

Original population	Irish			Kent		
	30			33		
1st generation	20.0 (2)	18.8 (4)	Not available	19.5 (2)	16.8 (4)	Not available
2nd generation	26.0 (2)	25.5 (4)	„	30.0 (2)	24.3 (3)	„
3rd generation	31.0 (2)	35.3 (3)	34.0 (2)	37.0 (2)	37.0 (3)	33.0 (1)
	Four-plt. groups	Two-plt. groups	One-plt. groups	Four-plt. groups	Two-plt. groups	One-plt. groups

(Figures in brackets indicate number of lines in each group.)

4. RESULTS: CHANGES IN GENETIC VARIATION

It now remains to look more closely at the changes in the pattern of genetic variation which occur under selection, in particular at the extent of release of variation from within the initial groups, and the maintenance of genetic variation within lines under the different mating systems.

(i) *Release of variation from within initial groups*

Changes in the *mean* performance of the lines have already been considered, but the *variance* is also important since it determines the range between the extreme early and late plants and thus the possible selection differential for further advance. Table 6 shows the mean range in 1957 for the original populations and for the lines derived

from, one, two and four initial parents ; in the single-plant lines data are only available for the last generation.

While the mean range of the first generation progenies is well below that of the original populations, by the third generation of selection the range between the extreme plants derived from even one individual is equal to, or exceeds, that of the original population. There is no difference in the third generation between the ranges derived from initial groups of one, two or four plants, nor between those from Irish and from Kent. Evidently, one plant can carry more potential variation than is expressed phenotypically in the original population.

(ii) *Maintenance of genetic variation within lines*

The genetic variance of the selection lines would be expected to decline because (i) selection for an extreme phenotype might select

TABLE 7
Mean variances within selection lines, 1957

Original population	Irish				Kent			
	18.7				40.0			
1st generation	19.3 (2)	19.3 (4)	Not available	Not available	13.8 (2)	13.7 (4)	Not available	Not available
2nd generation	20.3 (4)	16.6 (8)	15.7 (2)	„	16.1 (4)	20.1 (8)	31.6 (4)	„
3rd generation	21.6 (4)	23.4 (7)	19.9 (6)	13.7 (3)	15.6 (4)	22.5 (7)	11.9 (3)	41.1 (3)
	Fourway	Sib-crosses	Selfing/sib-crosses	Selfed lines	Fourway	Sib-crosses	Selfing/sib-crosses	Selfed lines

(Figures in brackets indicate the number of lines in the group.)

homozygotes for plus or minus alleles, and (ii) inbreeding would tend to bring about homozygosis. The phenotypic variances of the original populations and the mean variances of the lines derived from them under the different mating systems are given in table 7.

In Irish ryegrass there is no regular decrease in variance accompanying selection and/or inbreeding, except possibly for the third generation selfed lines. With this exception, the phenotypic variances in the third generation are equal to, or greater than, that of the original population. In Kent, the variance of the original population is higher than in Irish, and also than in most of the selection lines. There is again no regular decrease in variance from the first to the third generation, and in Kent the mean variance of the selfed lines in the third generation is greater than that of the original population. It is evident

that even under sib-mating or selfing, considerable phenotypic variation is maintained in these lines over the first three generations. The next section shows that there is no regular change in the environmental or within-plant variation during these generations of selection, and one may conclude that these figures for a high phenotypic variance reflect the maintenance of a high genetic variation also within the selection lines.

(iii) *Developmental stability of inbred and outbred material*

In many outbreeding organisms, inbred or homozygous material is less well buffered against environmental changes and shows a lower developmental stability than the heterozygotes (Lerner, 1954). In the present material the developmental error (within-plant variation) was calculated from replicated clones for the original population plants and for the parents of the first two generations of selection. Block differences were also calculated separately for the different mating systems to see whether the inbred material was more sensitive to environmental fluctuations between blocks.

As can be seen from table 8, there is no obvious change in the developmental error of clonal material after two generations of selfing, or of selfing followed by sib-mating, nor is there any regular change in the variance between blocks following inbreeding. The present mating systems have not led to any decrease in the developmental stability of the selection lines.

5. DISCUSSION

The present paper deals with the first three generations of selection only, and the results cannot be compared with those of long-term response to selection, which have been reviewed by Mather (1953a, 1955), Lerner (1950), and Robertson (1955). It will be sufficient at this stage to compare the genetic structure revealed in these strains of *Lolium* with that of other outbreeding species, such as *Drosophila melanogaster*, and also to discuss the plant breeding implications of such a genetic structure.

(i) *Population structure*

One of the most interesting findings of the present work is the great potential variation which is carried within single individuals of *Lolium perenne* and capable of being released in a small number of generations. Similar storage of genetic variation has been reported in most outbreeding species which have been studied in detail (Mather, 1953a, Falconer, 1955; Manning, 1955) and considerable response to selection is usually possible.

In the present work, however, response has not followed a symmetrical pattern, the advance towards lateness being rather greater than that towards earliness. The most obvious immediate cause of

the asymmetry is the unequal selection differential in the two directions (table 5), but the reasons for this are obscure and need further study. Scaling effects can be ruled out, since the variance is not related to the mean, and the environmental switch, photoperiod, varies linearly over most of the response range. Inbreeding depression, however, results in later heading, and selection for early heading might therefore favour heterozygotes. The early lines might thus show less response than the late ones.

TABLE 8
Effect of mating system on developmental error of replicated clones, 1957

	Irish				Kent			
	Mean ear emergence	v Error	v Block	No. of clones	Mean ear emergence	v Error	v Block	No. of clones
<i>Original population</i>	1.9 M	4.25	0.12	38	24.9 M	2.93	-0.04	34
<i>1st generation</i>								
Outcrosses	6.9 M	4.58	0.86	8	23.6 M	3.12	0.08	15
Selfings	4.5 M	3.31	0.25	9	2.6 J	3.09	0.03	5
<i>2nd generation</i>								
Fourway crosses	9.5 M	3.99	1.77	12	21.8 M	2.08	-0.02	15
Sib-crosses	6.8 M	2.68	0.25	14	24.1 M	1.73	0.49	15
Selfings/sib-crosses	4.6 M	2.71	0.37	10	4.9 J	2.94	-0.38	5
Selfed lines	6.3 M	3.80	0.52	6	17.2 M	4.37	-0.49	6

Specimen analysis of variance

	dF	SS	MS	Expectation
Genotypes	37	5352.26	144.68	$V_E + 5 V_G$
Blocks	4	35.60	8.90	$V_E + 38 V_B$
Error	148	628.80	4.25	V_E
Total	189	6016.66	...	...

$$\text{Hence } V_E = 4.25 \quad V_B = 0.12 \quad V_G = 28.09.$$

No selection limits have yet been reached, and would not be expected as early as the third generation. In some lines, however, both male and female fertility are decreasing, as found also by Mather and Harrison (1949) following selection for high and low chaeta number in *D. melanogaster*. This is more marked in the fourth generation, in which the parents selected for two lines (KLE and KEL) gave only nine and thirty-two seeds respectively and had to be replaced by less extreme plants.

In spite of the marked response to selection accompanied by inbreeding there has been little, if any, decrease in genetic variation

within these lines up to the third generation of selection, even in the closely inbred material. Several previous cases have been reported where selection or inbreeding have not led to the expected decline in genetic variation (Mather and Harrison, 1949 ; Lerner and Dempster, 1951 ; Manning, 1955) and this has usually been interpreted as due to natural selection against unbalanced gene combinations. Similar natural selection can be seen in the present work where most of the selfed lines died out, and there was also some mortality in the lines derived from selfing followed by sib-mating. This was due partly to self-sterility and partly low to vigour of the resulting seedlings, and selection for balanced heterozygotes might occur at both these stages. Such selection could also account for the absence of any increase in the developmental error of the inbred material.

Another possible mechanism for the maintenance of genetic variation is that of genotype/year or genotype/location interaction (Sprague, 1955 ; Robinson and Comstock, 1955). In field crops of ryegrass, for instance, the date of harvesting may vary with season or district, and thus affect the selective value of a particular heading date, while in the present experiment (tables 2 and 5) the heading dates of particular genotypes are affected differentially by yearly fluctuations in spring temperature.

The phenotypic variation within the selection lines will be influenced also by any changes in the developmental or environmental variation of the material during the progress of selection. The superior developmental stability (or homeostasis) of heterozygotes in outbreeding populations has been emphasised by Lerner (1954) but later studies suggest that the stability is not due to heterozygosity *per se*, but is under genetic control and is developed in the population under the action of selection (Mather, 1953*b* ; Jinks and Mather, 1955). In the present work, the inbred material shows no lower developmental stability for ear emergence than the outbred stocks, as measured either by the within-plant variance from replicated clones or by differences between blocks. The developmental stability of other ear characters (to be presented in a later paper) also shows little difference between the inbred and outbred material. It must be pointed out, however, that the plants surviving in the inbred material may well have been balanced heterozygotes selected for vigour.

One may conclude that the present populations of *L. perenne* show a similar genetic structure to that of other outbreeding organisms which have been studied in detail, in that most individuals are highly heterozygous and carry much potential genetic variation, which can be released quite rapidly for selection to act upon.

(ii) Plant breeding implications

The genetic structure of these outbreeding populations involving extreme heterozygosity of parents with considerable storage of genetic variation, contrasts with that of the self-fertilising cereals, in which

nearly all plants are homozygous, and most of the genetic variation is apparent in the phenotype. Many plant breeding concepts have been based on the situation in the cereals, and it is important to realise the difference in methods and outlook imposed by an outbreeding system.

Most of the genetic variation in a local population, or a bred strain, is hidden and it is selection rather than the potential content of the gene pool which determines the phenotypic range. It is sometimes thought that where a particular phenotype does not occur in an established strain, plant introduction or hybridisation is necessary to obtain it. While this may be true for certain major gene differences, such as disease resistance, it does not necessarily hold for polygenic characters. In the present experiment, for instance, by the fourth generation of selection for date of ear emergence, the full range of Irish for this character has been obtained from Kent, and that of Kent from Irish.

Pilot studies on such components of yield as leaf size, rate of leaf production and tillering have revealed a similar genetic structure for these characters, with considerable genetic variation within strains, and the response to selection for these characters is now under study. These findings suggest that, in a region such as Britain, where adapted forage species are already available, further improvement may be most usefully obtained by critical selection within adapted strains, rather than by the wide collection and introduction of new and unadapted ecotypes.

The genetic structure of the population also determines the optimum number of unrelated plants on which to base a strain. The difficulty is that too few basic plants may lead to inbreeding depression and loss of vigour in later generations of multiplication, while too many could result in excessive release of variation during multiplication.

In perennial ryegrass, Corkhill (1956) found that six parents were adequate, providing they showed high combining ability, while, earlier, Jenkin (1931) had suggested that eight unrelated plants were quite sufficient. The present results show that much genetic variation can be carried even within one individual, and that over the first three generations there is little difference in the rate of release from one, two or four parents. The results for ear characters (to be presented later) also indicate that for Irish and Kent ryegrass the fourway crosses are sufficient to maintain vigour for at least three generations of selection. These lines were continued using only four parents per generation, while in seed multiplication the full natural rate of increase is allowed. It seems likely therefore that four plants of similar phenotype would be quite adequate as the basis of a herbage grass strain.

Finally, the population structure influences the techniques of seed multiplication. In perennial ryegrass, even a carefully selected strain, based on a few parents of similar phenotype, will contain considerable potential genetic variation, and any selective action of seed multiplication can therefore result in genetic shift.

The problem of change in seed stocks during multiplication in regions other than the district of origin has been reviewed by Beard and Hollowell (1952). In the U.S.A., greater seed yields are obtained in the dry south-western states, such as Arizona and California, than in the humid north-east, but considerable changes in performance were reported in bluestems, blue grama, side-oats grama, switchgrass and brome-grasses when grown 300-400 miles north of the seed-producing areas.

Even where differences in climate are small, the selective effects of management, including such factors as the latest date of grazing in the spring, or date of cutting the seed-crop, may be important. In the late-flowering S.23 perennial ryegrass, for instance, a shift towards earliness of ear emergence during seed multiplication has been reported by Griffiths (1951), while Davies (1954) in this strain found an average change in mean heading date of 11.3 days between first and sixth generation stocks. More detailed figures for particular generations of multiplication have been given by Cooper (1959c) who found that up to five generations of multiplication could occur with only a slight shift in the date of heading. No corresponding changes in winter requirement before heading, or in leaf size, rate of leaf appearance or tillering in the seedling stage could be detected, although ample additive genetic variation for these characters exists in the strain.

It is evident that the mere existence of genetic variation in a strain does not necessarily lead to changes during seed multiplication. Whether these occur will depend on the direction and intensity of selection associated with the techniques of multiplication. In out-breeding species, such as perennial ryegrass, it is not possible to eliminate genetic variation in the initial stocks, and seed multiplication schemes are designed to minimise any changes between the foundation stocks and the certified seed as received by the farmer. Such schemes in the herbage grasses usually involve clonal propagation of foundation parents, and limitation of the number of generations of multiplication. An important addition to this list might be the use of a system of management which maintains the strain within the required phenotypic range.

6. SUMMARY

1. This paper describes the response to selection for date of ear emergence in Irish and Kent perennial ryegrass during the first three generations. The experiment was designed to compare the effects of different mating systems and different initial population sizes, and also to assess the genetic variation carried within small groups of parents.

2. Response has continued up to the third generation of selection in all lines and there is little difference in the rate of advance between the lines derived from one, two or four initial parents. Kent shows more rapid response than Irish, and in both strains the early parental

groups respond more rapidly than the late. In all cases, advance is asymmetrical, the shift towards lateness being greater than that towards earliness. By the fourth generation, the full range of Irish has been obtained from within Kent, and that of Kent from within Irish.

3. After three generations of selection, the range between the extreme early and late plants derived from a single individual is greater than that of the original population. One plant can evidently carry more potential genetic variation than is expressed phenotypically in the initial population.

4. Considerable genetic variation is maintained within the selection lines even with close inbreeding, and there is no increase in the developmental error within genotypes, nor in the fluctuations between blocks, following inbreeding.

5. It is concluded that *Lolium perenne* shows a similar genetic structure to that of other outbreeding organisms which have been studied in detail, such as *Drosophila melanogaster* and the mouse, in that most individuals are highly heterozygous and carry much potential genetic variation. The comparative uniformity of phenotype results from the limiting action of selection rather than from the absence of genetic variation.

6. The plant breeding implications of such a genetic structure are discussed, with particular reference to the optimum number of parents as the basis of a strain, and the problem of genetic shift during seed multiplication.

Acknowledgments.—It is a pleasure to acknowledge the facilities provided and interest taken in this work by Professor E. T. Jones, former Director, and Professor P. T. Thomas, present Director of the Welsh Plant Breeding Station. I am grateful to Mr A. R. Beddows, Dr M. Borrill and Dr E. L. Breese of the Grass Breeding Section of the Station for helpful discussion, and to Miss Irene D. Rees for valuable assistance with the manuscript.

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