SELECTION AND POPULATION STRUCTURE IN *LOLIUM*

I. THE INITIAL POPULATIONS

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1. INTRODUCTION

IN out breeding crops, such as most of the herbage grasses, the breeding system poses two related problems ; firstly, the existence of pronounced inbreeding depression, often associated with some degree of self-incompatibility, makes it difficult to base an improved strain on one individual ; and secondly, the heterozygosity of parent plants may result in the release of variation during seed multiplication. From the plant breeding point of view, it is important to know the amount of genetic variation carried within a small group of plants and its rate of release under selection, and also the minimum size of initial population necessary to maintain vigour and avoid inbreeding depression.

The aim of the present work was to study the genetic structure of certain ryegrass populations with respect to one agronomic character, the timing of inflorescence development. It is hoped that this work will assist in the effective planning of selection and breeding programmes and also serve as a pilot study for other agronomic characters in the herbage grasses.

(i) Choice of character

Inflorescence development was chosen for several reasons. It is of great agronomic and adaptive importance in controlling the duration of the life-cycle and the season of flowering. A certain amount is known about its developmental basis ; most populations possess a "winter requirement" for short-day and/or low temperature before floral induction can occur, followed by a requirement for long photoperiod for floral initiation and elongation (Cooper, 1951, 1957). These developmental responses are under polygenic control and vary *between* and *within* populations (Cooper, 1954), and finally, standard field and glasshouse techniques are available for their measurement (Cooper, 1956, 1957).

(ii) Choice of material

The out breeding species of *Lolium* (*L. rigidum*, *L. italicum* and *L. perenne*) are all diploid ($2n = 14$) and interfertile (Jenkin, 1954*a*, *b*, *c*). They have a geographical range in Eurasia from the Western Himalayas and the Caucasus through the Mediterranean region to North-west Europe as far north as South Norway. They have also been introduced

as forage plants or weeds to most temperate and Mediterranean climatic regions.

Three contrasting populations were chosen to cover the range of heading behaviour in the group, namely (a) the early-flowering winter-annual *Wimmera* ryegrass (*L. rigidum* Gaud.) from South Australia ; (b) the early-flowering hay strain of perennial ryegrass (*L. perenne* L.) Irish commercial ; (c) the late-flowering pasture strain of perennial ryegrass, Kent indigenous. These are all local strains which have developed under the selective action of different climates and managements. Their agronomic behaviour and past selection histories are well documented (Beddows, 1953) and will be discussed later.

(iii) Sequence of study

The present paper attempts to assess the variation *between* and *within* these initial populations, as influenced by environmental and seasonal fluctuations ; and also to see how far the differences between populations in heading behaviour can be related to differences in the selective action of the climates and managements under which they have evolved. A later paper will deal with the partition of the variation within these populations into environmental and genetic components, and the further separation of the genetic variation into additive and non-additive portions, using estimates from clonal replicates and from parent-progeny regression.

The effects of selection for early and late ear emergence over three generations, starting with different population sizes exposed to different intensities of inbreeding will also be reported. The primary object was to assess the potential genetic variation carried within one, two or four individuals, and its rate of release under selection. At the same time, changes in other ear characters were studied to detect any correlated response and to measure the effect of the degree of inbreeding. These results, to be reported later, throw light on the important problem of the number of foundation plants required to maintain vigour and avoid inbreeding depression.

The present paper describes firstly, the heading behaviour of the initial populations in the field after both autumn and spring sowing, and then examines the developmental responses which underlie these field differences.

2. HEADING BEHAVIOUR IN THE FIELD : AUTUMN SOWING

Inflorescence development in the spring after overwintering outdoors is controlled largely by photoperiod, although the rate of inflorescence elongation is modified by spring temperature. The usual criterion recorded in the herbage grasses is the date of ear emergence, defined as that date when the tip of the third head on each plant emerges from the leaf sheath (Jenkin, 1931). The seasonal variation in ear emergence for *Wimmera*, Irish and Kent ryegrass is given in tables 1, 2 and 3 respectively, the years being arranged in order of

TABLE 1

Seasonal variation in ear emergence at Aberystwyth. Wimmera ryegrass. All autumn-sown, overwintered outdoors, except where otherwise stated

Year	Ear emergence			Accum. Temp. (March-April)
	Mean	Var.	No. of plants	
1957	24.0 Apr.	42.3	195	458
1948	30.4 Apr.	28.1	30	487
1949	3.9 May	16.7	20	350
1956	6.5 May	8.2	20	278
1958	7.3 May	19.3	23	223
1955	10.2 May	29.7	50	257
1947	13.2 May	5.6	47	238
1949 (Unheated house)	19.4 Apr.	13.8	20	...
(Heated house)	16.0 Apr.	17.3	20	...

All one stock ex Waite Agricultural Institute, Adelaide.

TABLE 2

Seasonal variation in ear emergence at Aberystwyth. Irish perennial ryegrass. All autumn-sown, overwintered outdoors

Year	Ear emergence			No. of stocks	No. of plts.	Accum. Temp. (March-April)
	Mean of stocks	Range of means	Mean var.			
1957	28.9 Apr.	(28.1-30.3)	22.5	3	287	458
1956	8.0 May	(6.7-9.4)	23.0	4	184	278
1958	9.1 May	(7.5-9.4)	20.2	5	250	223
1954	9.6 May	(8.0-10.4)	26.9	8	234	257
1947	12.0 May	(11.7-12.3)	11.7	2	80	253
1955	18.0 May	...	30.7	1	50	239

TABLE 3

Seasonal variation in ear emergence at Aberystwyth. Kent perennial ryegrass. All autumn-sown, overwintered outdoors

Year	Ear emergence			No. of stocks	No. of plts.	Accum. Temp. (April-May)
	Mean of stocks	Range of means	Mean var.			
1957	22.2 May	(20.8-23.2)	44.6	3	292	495
1956	24.4 May	(22.5-27.1)	26.5	6	268	450
1954	24.4 May	...	26.4	1	125	464
1958	29.0 May	(25.6-31.0)	21.3	5	176	460
1955	1.7 June	...	24.5	1	50	446

earliness. All the material had been sown in the previous summer and overwintered as spaced plants in the field. These tables also include the accumulated temperatures above 42° F. for March and April for Wimmera and Irish, and for April and May for Kent, that is approximately for the two months prior to ear emergence.

The heading and temperature data up to and including 1950 refer to Penglais, Aberystwyth, 250 ft. above sea-level on a west-facing slope overlooking the sea. From 1954 onwards the data are taken from Gogerddan, Aberystwyth, 120 ft. above sea-level, but in an inland valley 3 miles from the sea, and liable to temperature inversions at night (Howe, 1953).

In Wimmera ryegrass, the date of ear emergence is comparatively early, but appreciable year-to-year variation occurs, from 24th April in a warm spring (1957) to 13th May in a cold spring (1947). Table 1 also gives the heading behaviour of Wimmera seedlings sown in the autumn of 1948 and overwintered in heated and unheated greenhouses. The higher temperatures in the greenhouses have accelerated ear emergence, in the heated house, by 18 days. As all the Wimmera seed came from the same source (the Waite Agricultural Institute, Adelaide), no comparison of different stocks is possible.

In Irish ryegrass, the date of ear emergence is again comparatively early, varying from 28th April in a warm spring (1957) to 18th May in a cold spring (1955). In Kent ryegrass, by contrast, ear emergence is late, ranging from 22nd May in 1957 to 2nd June in 1955. The year-to-year variation is not, however, as great as in Wimmera or Irish. The samples of Irish and Kent ryegrass tested represent ordinary commercial stocks, and in tables 2 and 3 the results from different stocks in the same year are bulked. Table 4, however, gives a comparison of three stocks of Irish and three of Kent for one early year (1957) and one late year (1958). In both Irish and Kent, differences between stocks are very small compared to the difference between the two strains. These findings are supported by the more detailed records of Beddows (unpublished) on the seasonal variation in ear emergence of a wide range of species and strains of herbage grasses.

Estimates of the error variance of single spaced plants derived from clonal replicates of Irish and Kent ryegrass in 1955 to 1958 are given in table 5. No significant differences were detected between blocks in the same field in any of these years and it is evident that, within both strains, the greater part of the variation is genetic.

Inspection of tables 1, 2 and 3 indicates that for Wimmera and Irish ryegrass and to a lesser degree for Kent, there is a close relation between spring temperature and the earliness of ear emergence. Previous transplant studies (Cooper, 1952) using clonal material of early-, medium-, and late-flowering perennial ryegrass showed a good correlation between the date of ear emergence within each group and the accumulated temperature above 42° F. during inflorescence development. In the present work, therefore, the regressions of date of

ear emergence on accumulated temperature for a number of years were calculated, using March and April temperatures for Wimmera and Irish, and April and May temperatures for Kent ryegrass (fig. 1).

TABLE 4

*Variation between stocks in ear emergence. Irish and Kent perennial ryegrass.
All autumn-sown, overwintered outdoors*

Strain	Stock	Ear emergence	
		1957	1958
Irish	Ba 6650 Ba 6738 Ba 6773	30.3 Apr. 28.4 Apr. 28.1 Apr.	9.4 May 8.1 May 7.5 May
	Mean	28.9 Apr.	8.3 May
Kent	Ba 6565 Ba 6622 Ba 6740	20.8 May 22.8 May 23.2 May	25.6 May 29.3 May 29.7 May
	Mean	22.3 May	28.2 May

Differences between stocks, non-significant in Kent :
significant at $P = 0.05-0.01$ in Irish.

In Irish and Wimmera, the regressions are highly significant and more than 60 per cent. and 70 per cent. respectively of the year-to-year variation can be attributed to accumulated temperatures. In Kent,

TABLE 5

Error variation in ear emergence from replicated clones. Irish and Kent ryegrass

Year	Error variance of single plant		Error variance of mean of 50 plants	
	Irish	Kent	Irish	Kent
1955	2.75	1.25	0.055	0.025
1956	2.86	3.02	0.057	0.060
1957	4.25	2.93	0.085	0.059
1958	2.86	2.81	0.057	0.056

where there is less year-to-year variation, the regression is barely significant, accounting for less than 20 per cent. of the seasonal variation. In this strain, by the time the photoperiodic threshold for floral initiation has been reached, spring temperature is probably no longer limiting.

From these results, one may conclude that in overwintered material of Wimmera, Irish and Kent ryegrass, all plants within each strain head fairly uniformly, Wimmera and Irish at the beginning of May

and Kent in late May. Considerable year-to-year variations occur, these being attributable largely to differences in spring temperature, once the critical photoperiod has been reached. In all strains there is a tendency for a warm spring both to accelerate heading and also to increase the variance. The greater part of the variation within samples is genetic; slight differences between stocks may occur in both Irish and Kent.

FIG. 1.—Effect of spring temperature on inflorescence development in Wimmera, Irish and Kent ryegrass.

$$\begin{array}{lll} \text{Wimmera} & . & Y = 25.0 - 0.059 (\pm 0.016)X \\ \text{Irish} & . & Y = 26.4 - 0.064 (\pm 0.015)X \\ \text{Kent} & . & Y = 42.3 - 0.031 (\pm 0.015)X \end{array}$$

(Y = date in May, and X = accumulated temperature.)

3. HEADING BEHAVIOUR IN THE FIELD : SPRING SOWING

When seedlings are overwintered outdoors, all the plants produce heads the following spring, and the range in ear emergence within each strain is comparatively small. The date of ear emergence is, in fact, a valuable agronomic character for separating strains into different maturity groups (Hawkins, 1950; Thomson and Seaton, 1956).

When sowing is delayed until the spring, the picture is rather different, as can be seen from tables 6 to 8 which show the effects of spring sowing on Wimmera, Irish and Kent respectively.

In Wimmera, all seedlings produce heads even when sown as late as mid-April, but sowing after the end of January leads to an increasing

TABLE 6

Effect of date of sowing on ear emergence at Aberystwyth. Wimmera ryegrass

Year of heading	Sowing date	Ear emergence		
		Mean	Var.	No. of plts.
1947	15 June 46	13.2 May	5.64	47
	11 Feb. 47	22.0 May	141.0	30
	15 Apr. 47	10.8 July	447.2	29
1949	15 Oct. 48	3.9 May	16.7	20
	29 Jan. 49	17.4 May	53.9	20
	29 Mar. 49	3.3 July	241.1	20
1955	23 Aug. 54	10.2 May	29.7	50
	7 Jan. 55	21.0 May	28.5	46
	3 Mar. 55	25.6 June	477.0	49
1957	8 Aug. 56	24.3 Apr.	31.6	95
	30 Jan. 57	25.2 May	196.0	41

All seedlings head in all sowing dates.

TABLE 7

Effect of date of sowing on ear emergence at Aberystwyth. Irish perennial ryegrass

Year of heading	Sowing date	Percentage heading	Ear emergence		
			Mean (or median)	Var.	No. of plts.
1947	15 June 46	100.0	14.4 May	8.6	89
	2 Nov. 46	100.0	11.8 May	10.4	40
	20 Jan. 47	95.0	(1 June)	...	40
	11 Feb. 47	66.7	(28 July)	...	60
	21 Feb. 47	67.5	(8 Aug.)	...	40
	20 Mar. 47	32.5	...	...	40
	1 Apr. 47	10.0	...	...	120
1949	15 Oct. 48	100.0	12.3 May	13.0	20
	29 Jan. 49	80.0	(8 July)	...	20
	29 Mar. 49	10.0	...	...	20
	25 Apr. 49	nil	...	...	60
1955	23 Aug. 54	100.0	17.9 May	30.7	50
	7 Jan. 55	100.0	2.4 June	16.2	49
	3 Mar. 55	45.4	...	...	44
	27 Apr. 55	nil	...	...	120
1956	12 Aug. 55	100.0	8.7 May	12.4	43
	5 Jan. 56	93.3	(7 June)	...	90
	13 Mar. 56	40.0	...	...	90
	8 May 56	1.1	...	...	90
	6 July 56	nil	...	...	90
1957	13 Aug. 56	100.0	28.1 Apr.	25.6	100
	30 Jan. 57	58.8	(30 June)	...	34

The same stock was used within any one year.

delay in the mean ear emergence date (up to mid-July following an April sowing) and also to a greatly increased range and variance.

In Irish and Kent ryegrass, a progressive delay in sowing after January not only leads to a delay in ear emergence, but also to a regular decrease in the proportion of plants which head (fig. 2). In

TABLE 8

Effect of date of sowing on ear emergence at Aberystwyth. Kent perennial ryegrass

Year of heading	Sowing date	Percentage heading	Ear emergence		
			Mean (or median)	Var.	No. of plts.
1947	15 June 46	100.0	29.4 May	24.0	30
	11 Feb. 47	15.0	...	...	60
	1 Apr. 47	nil	...	...	30
	17 Apr. 47	nil	...	...	30
1949	15 Oct. 48	100.0	28.7 May	18.2	20
	29 Jan. 49	50.0	21 Aug.	...	20
	29 Mar. 49	nil	...	...	20
	25 Apr. 49	nil	...	...	30
1955	23 Aug. 54	100.0	1.7 June (17 June)	24.5	50
	7 Jan. 55	96.0	...	...	50
	3 Mar. 55	12.2	...	...	49
	27 Apr. 55	nil	...	...	50
1956	12 Aug. 55	100.0	24.3 May	31.0	40
	8 May 56	nil	...	...	90
1957	13 Aug. 56	100.0	22.8 May	40.0	94
	30 Jan. 57	31.6	...	...	38

The same stock was used within any one year.

Irish ryegrass very few plants produce heads when sown after the middle of April, while in Kent the critical date is earlier, about the end of March. For any particular sowing date from January to April, the proportion of plants which form heads is higher in Irish than in Kent. Although fig. 2 is constructed from the results of several years, the decrease in heading with advancing sowing date is very regular, suggesting that the degree of winter induction provided by a particular sowing date is fairly uniform from year to year.

Within each strain, however, there is a range in the rapidity with which floral induction occurs after a spring sowing. A more detailed picture of this variation is given in fig. 3 which shows the distribution of ear emergence in Irish ryegrass after a series of sowings from autumn 1946 to summer 1947. After an autumn sowing all plants headed fairly uniformly as shown by the steep and linear probit line ; as the

FIG. 2.—Effect of sowing date on inflorescence development in Wimmera (●), Irish (X), and Kent (Δ) ryegrass.

FIG. 3.—Effect of sowing date on inflorescence development in Irish perennial ryegrass.
X 2

sowing date becomes later an increasing number of plants show a delay in heading and eventually fail to head at all. Spring sowing therefore reveals differences between genotypes in "winter requirement" for short-day and/or low temperature before floral induction. At the one extreme there are plants which fail to become induced when sown at the end of January, while at the other end of the range, some will head when sown in mid-April. The genetic control of these differences in inductive requirement will be considered in a later paper.

These results suggest that in the field material, while date of heading after overwintering is a measure of response to photoperiod modified in its expression by spring temperature, the extent of heading after a spring sowing is a measure of the winter requirement for short-day and/or low temperature. For both responses considerable differences between and within strains can be detected.

4. DEVELOPMENTAL RESPONSES : FLORAL INDUCTION

While these field sowings can distinguish between strains and also detect variation within strains, laboratory and greenhouse tests provide more rapid and reproducible results and also give information on the developmental responses to temperature and photoperiod which underlie the differences in field behaviour. The three initial strains, Wimmera, Irish and Kent have therefore been tested for the flowering responses under controlled conditions.

Earlier studies (Cooper, 1957) had shown that Wimmera ryegrass had no obligatory requirement for low temperature before floral induction, although in many plants heading could be accelerated by cold treatment. In Irish and Kent ryegrass, on the other hand, most plants had an obligatory requirement for low temperature, this being greater for Kent than for Irish. In all strains, considerable genetic variation in response to low temperature occurred within the strains. Later work, however, has suggested that short-day can also bring about floral induction, so these greenhouse tests were designed to include both short-day and low temperature treatments.

(i) *Response to induction : August 1956*

The following inductive treatments were given to Wimmera, Irish and Kent ryegrass :

- (a) low temperature alone ($0-4^{\circ}\text{C.}$) given to the germinating seeds (for details see Cooper, 1956) ;
- (b) short-day alone (8 hr. photoperiod) given to the young seedlings in a warm greenhouse.

Each treatment was given for 4 and 8 weeks, and after induction all material was transferred, on 1st August 1956, to continuous light in a warm greenhouse. The untreated controls were sown to germinate on the date of transfer.

Leaf number on the main shoot before heading was recorded for each seedling, while for the short-day treatments the leaf number at transfer to continuous light was also recorded, and the leaf number between transfer and heading calculated as the measure of reproductive development. Seedlings which had not headed by the 20th leaf stage were recorded as non-heading.

The results are given in table 9. Wimmera ryegrass showed no obligatory requirement for either short-day or low temperature but in the controls the leaf numbers ranged from 6 to 18 with a mean of 11.2 and a

TABLE 9
Response to low temperature and short-day induction. All transferred to continuous light 1st August 1956

Strain	Exposure (wks.)	Low temperature			Short-day (8 hr.)		
		Percentage heading	Leaf no.		Percentage heading	Leaf no.	
			Mean	Var.		Mean	Var.*
Wimmera	0	100.0	11.2	6.4	100.0	11.2	6.4
	4	100.0	5.5	1.6	100.0	8.0	8.1
	8	100.0	4.3	0.2	100.0	4.1	1.2
Irish	0	11.8	...	...	11.8	...	...
	4	19.2	...	...	25.6	...	...
	8	81.3	(7.9)	...	27.0	...	...
Kent	0	nil	...	...	nil	...	...
	4	4.0	...	...	16.0	...	...
	8	25.7	...	...	30.0	...	...

* Leaf no. after transfer. Each sample based on 80 initial seedlings.

For leaf numbers, significant difference at $P = 0.05$ is 0.72.

For percentage heading, significant difference at $P = 0.05$ varies from 5.0 per cent. at the 0 and 100 per cent. levels to 22.5 per cent. at the 50 per cent. level.

variance of 6.45. Four weeks' low temperature resulted in complete induction in nearly all seedlings and after 8 weeks' treatment the leaf number was reduced to 4 or 5 in all plants. Four weeks of short-day also had an inductive effect, though not as great as the same period of cold, and 8 weeks' short-day was enough for complete induction.

In Irish ryegrass, 11.8 per cent. of the seedlings headed without any inductive treatment, but even 8 weeks of cold induced heading in only 81.3 per cent. of the plants. Again the inductive effect of short-day was not as great as the same period of low temperature.

In Kent, on the other hand, no plants produced heads without short-day or low temperature induction, but the effect of short-day was equal to that of cold. Kent evidently has a greater requirement for induction than Irish, and only 25 per cent. to 30 per cent. of the plants headed after 8 weeks' treatment in this experiment.

These results, while emphasising the difference between the annual Wimmera and the two perennial strains, also reveal differences within

strains in response to induction. In Irish, for instance, a few plants are able to head without either short-day or cold, while others require more than 8 weeks' exposure.

(ii) *Response to induction : January 1958*

This first experiment did not provide complete induction in the perennial strains and the test was therefore repeated using longer

TABLE 10
Response to low temperature and short-day induction. All transferred to continuous light on 30th January 1958

Strain	Exposure (wks.)	LT alone			LT+SD			SD alone		
		Per cent. heading	Leaf no.		Per cent. heading	Leaf no.		Per cent. heading	Leaf no.*	
			Mean	Var.		Mean	Var.		Mean	Var.
Wimmera	0	100.0	10.7	17.97	100.0	10.7	17.97	100.0	10.7	17.97
	4	100.0	4.8	0.48	100.0	5.4	1.31	100.0	5.1	0.54
	8	100.0	5.0	0.45	100.0	4.8	0.25	100.0	4.6	0.39
	12	100.0	5.0	0.50	100.0	4.3	0.28	100.0	4.6	0.69
Irish	0	42.5	...	...	42.5	...	...	42.5	...	...
	4	57.5	(16.3)	...	48.7	...	...	90.0	(7.7)	...
	8	94.7	(10.0)	...	97.5	(8.0)	...	100.0	4.7	0.57
	12	100.0	10.0	5.09	100.0	8.5	3.13	100.0	4.2	1.45
Kent	0	7.5	...	...	7.5	...	...	7.5	...	...
	4	48.4	...	...	12.5	...	...	84.2	(9.4)	...
	8	82.2	(11.4)	...	94.7	(9.2)	...	100.0	5.3	1.42
	12	100.0	11.6	5.73	100.0	9.3	2.65	100.0	5.1	1.34

* Leaf no. after transfer. Each sample based on 40 initial seedlings.

For leaf no., significant difference at $P = 0.05$ is 0.96.

For percentage heading, significant difference at $P = 0.05$ varies from 18 per cent. at the 0 and 100 per cent. levels to 26 per cent. at the 50 per cent level.

inductive periods, and also applying short-day and low temperature concurrently. The following treatments were given to Wimmera, Irish and Kent ryegrasses: (a) low temperature alone ($0.4^{\circ}\text{C}.$); (b) short-day alone (8 hr. photoperiod) and (c) short-day and low temperature concurrently. In the last case, the seedlings were grown in vermiculite until the first leaf emerged, then transferred to the refrigerator and given 8 hr. photoperiod, using four 15-watt "daylight" fluorescent tubes (intensity at seedling level, 90 ft. candles).

Each treatment was given for 4, 8 and 12 weeks, and the sowings arranged so that all material was transferred to continuous light in a warm greenhouse on the same date, 30th January 1958. The untreated controls were sown to germinate on this date. Records of leaf number before heading were taken as in the previous experiment.

The results of these induction treatments (table 10) confirmed the

earlier findings and also showed that low temperature plus short-day had slightly more effect than low temperature alone. Wimmera again proved to have no obligatory requirement for short-day or cold, but the controls showed a range of leaf numbers from 5 to 23 with a mean of 10.7. Four weeks of low temperature alone, or of short-day alone, or of both, were sufficient for complete induction with reduction of the leaf number to 4 or 5. Further treatment had no additional effect.

FIG. 4.—Effect of low temperature induction on inflorescence development in Irish perennial ryegrass.

In Irish, a few plants headed without any inductive treatment, but 12 weeks of low temperature alone and 8 weeks of short-day alone were necessary for complete induction in all plants. The combination of short-day and low temperature was slightly more effective than low temperature alone, but not as effective as short-day alone. Unlike Wimmera, even after 12 weeks' short-day plus cold, the leaf numbers in Irish were not uniform, but ranged from 5 to 12, with a mean of 8.5. Following either 8 or 12 weeks' exposure to short-day alone, however, the leaf number after transfer was very uniform, being either 4 or 5.

It seems that even after complete induction with short-day plus cold, there is still genetic variation within the strain for minimum leaf number.

In Kent, only one seedling headed without cold or short-day, and the requirement for complete induction was rather higher than in Irish. The combination of cold and short-day was again more effective than cold alone. As in Irish, even 12 weeks of short-day plus cold gave a range in leaf numbers from 6 to 13 with a mean of 9.3, contrasting with very uniform heading after 12 weeks of short-day alone.

The differences within strains in response to induction are further illustrated in fig. 4 which gives probit diagrams for heading behaviour in Irish ryegrass under cold induction alone. A few plants can initiate heads without any low temperature while others require more than 8 weeks' cold treatment. A similar range occurs in Wimmera and Kent ryegrasses and also for response to short-day induction. Partial induction treatments evidently reveal considerable genetic differences within the populations, in contrast to their more uniform behaviour when fully induced.

While the strains show the same relative behaviour in these two greenhouse tests, the numerical values for the proportion of heading plants and for leaf number before heading are not the same. This applies particularly to the treatments with short-day alone, and differences in greenhouse temperatures during the short-day exposures may be responsible for these differences in response.

These results not only reveal the developmental basis of differences between strains in heading behaviour in the field after spring sowing, but also show that considerable genetic differences in inductive requirement occur within each strain, differences which are not apparent in the usual environment where all material receives enough cold or short-day for complete induction.

5. DEVELOPMENTAL RESPONSES : FLORAL INITIATION

In order to assess the requirement for long photoperiods for floral initiation, it is first necessary to ensure that all the material is fully induced. This can be done either by exposing it to winter conditions outdoors, or by giving enough controlled short-day or low temperature for this purpose.

The first of these methods has been used to test the photoperiodic response of Wimmera, Irish and Kent ryegrass. Seeds were sown in autumn 1948, and the seedlings were transferred to natural photoperiods outdoors soon after germination. In January 1949 the following photoperiod treatments were imposed : (a) 9 hr. day throughout ; (b) 11 hr. day, as soon as the natural day length reached 11 hr. and (c) natural day length throughout.

Both leaf number before heading and date of ear emergence were recorded as measures of reproductive development. The results for

all strains are given in table 11, while the differences *within* Irish are further illustrated in fig. 5.

In Wimmera, all plants headed in spring and summer 1949. There was no difference in leaf number, *i.e.* in time of floral *initiation* between natural day length and 11 hr. photoperiod, but the 9 hr. photoperiod resulted in an average delay of just over one leaf stage with a range from 12 to 18 leaves and an increased variance. Ear emergence was delayed

TABLE 11

Response to photoperiod in strains of Lolium. All sown 15th October 1948, overwintered outdoors

		Natural day length	11 hr. day	9 hr. day
Wimmera . .	Percentage heading	100.0	100.0	100.0
	Date of heading { Mean Var.	3.9 May 16.7	4.4 June 45.2	19.4 June 177.6
	Leaf no. { Mean Var.	12.9 0.81	12.8 1.17	14.1 2.94
Irish . . .	Percentage heading	100.0	95.0	35.0
	Date of heading { Mean Var.	12.3 May 13.0	(11 June) ...	
	Leaf no. { Mean Var.	12.1 0.41	(11.9) ...	
Kent . . .	Percentage heading	100.0	5.0	None
	Date of heading { Mean Var.	11.0 June 18.2		
	Leaf no. { Mean Var.	14.5 0.67		

All figures based on 20 seedlings.

by a month in the 11 hr. photoperiod compared to natural day, an effect presumably on the rate of inflorescence *elongation*. In the 9 hr. photoperiod ear emergence was still further delayed, with a greatly increased variance; some plants produced heads as rapidly in 9 hr. day as in natural day lengths, while others were delayed for 8-9 weeks. There is evidently considerable genetic range in response to photoperiod within the population, both for floral initiation and for subsequent elongation.

In Irish, all plants produced heads uniformly in natural photoperiods but only 95 per cent. of the seedlings headed when the photoperiod was reduced to 11 hr. although the median leaf number remained unchanged; the median date of ear emergence was, however, almost a month later than in natural day. Most plants evidently *initiated*

heads as rapidly in 11 hr. day as in natural day lengths, but elongation of the inflorescence was delayed. In the 9 hr. photoperiod, only 35 per cent. of the seedlings headed and even the earliest of these was considerably delayed in both leaf number and ear emergence. In Irish ryegrass also genetic variation in response to photoperiod is carried between plants in the population, but is only revealed at the threshold photoperiods.

In the late-flowering Kent, all seedlings produced heads uniformly in natural photoperiods, but only one plant headed in the 11 hr. photoperiod and none in the 9 hr. The critical day length for nearly

FIG. 5.—Effect of photoperiod on inflorescence development in Irish perennial ryegrass.

all plants is evidently above 11 hr., and the present treatments cannot therefore detect any genetic variation.

These results, while indicating the differences in photoperiodic requirements *between* the three strains, Wimmera, Irish and Kent, also emphasise the genetic variation *within* the strains, variation which is not apparent in the usual environment but which is revealed at threshold exposures.

6. DISCUSSION

The three strains Wimmera, Irish and Kent differ in their heading behaviour in the field, both when autumn and spring sown. These variations in field performance can be traced to genetic differences, firstly in the "winter requirement" for short-day or low temperature before floral induction can occur and, secondly, in the photoperiodic requirements for floral initiation and elongation. These results pose two problems for discussion; firstly, the relation between the heading

responses of each strain and the climatic and agronomic selection under which it has evolved and, secondly, the maintenance of genetic variation *between* plants in a phenotypically uniform population. These topics will be discussed for each strain separately.

(i) *Wimmera ryegrass*

Wimmera ryegrass, a winter-annual strain, was first recorded from the Wimmera wheat-growing region of Victoria, Australia (34-36° S.) but it is now grown in West and South Australia and in New South Wales also. It was reputedly introduced from Europe in 1887 (Drake, 1931) and has thus undergone more than 60 generations of selection under Australian conditions. It is similar in morphology and life-cycle to the annual *L. rigidum* from the Mediterranean region, and may have been derived from this source.

The Wimmera region has a semi-arid Mediterranean climate with a January temperature of 60° to 70° F., a July temperature of around 50° F., and a mean annual rainfall of 10-15 in. mainly in the winter. The photoperiod (civil twilight) varies regularly from a minimum of 10¾ hr. in June to a maximum of 15½ hr. in December. Fig. 6 shows the seasonal distribution of photoperiod and the water-balance (according to Thornthwaite's formula) for a similar climate in Adelaide, Victoria. I am indebted to Dr Melvyn Howe, of the Department of Geography, U.C.W., Aberystwyth, for the water-balance diagram (Howe, 1958).

It is evident that the region has a severe water deficit, lasting from about the end of November to the beginning of May when the autumn rains begin. The growing season, as limited by water supply, thus extends from early May to the end of November. In practice (Donald, 1954) Wimmera ryegrass germinates with the autumn rains, grows vegetatively during the winter and initiates heads before the end of September, *i.e.* in photoperiods of less than 13 hr. Ear emergence follows at the end of October and seed must be formed before the water supply is exhausted at the end of November. The plant then dies, the seed is shed and germinates with the autumn rains in the following May. In this way the strain, although annual, persists regularly from year to year.

This limited growing season selects genotypes in which all tillers become fully induced before the end of September, *i.e.* with mean temperatures rarely less than 50° F. and with photoperiods not much below 11 hr., and which can also initiate heads by this date, *i.e.* in photoperiods less than 13 hr. However, the longer the period of vegetative growth, the more tillers and the more seed will be produced per plant, so one would expect quantitative selection for the later end of this range. This, in fact, corresponds to the range in response to winter induction and to photoperiod which is revealed in the present tests. Selection is evidently not for one sharply defined response, but

for any of a wide combination of flowering responses which allow of survival and perpetuation of the plant.

Similar life-cycles and developmental responses have been recorded by Aitken (1955*a, b*) in subterranean clover (*Trifolium subterraneum*)

FIG. 6.—Life-cycle of Wimmera ryegrass in relation to water-balance and photoperiod at Adelaide, S. Australia.

— — — — — Potential Evapo-transpiration.
 - - - - - Precipitation.

which is grown in association with Wimmera ryegrass in the same climatic regions. She has also been able to show that varietal differences in adaptation to the length of the growing season can be related to genetic differences in winter requirement for floral induction and in response to long photoperiod for floral initiation.

(ii) *Irish perennial ryegrass*

Irish ryegrass is grown for seed in Northern Ireland (55° N.) where more than 100,000 acres are harvested annually. According to Mercer (1929) the growing of ryegrass for seed was introduced about 1830-40 from Ayrshire to Banbridge, County Down. A similar system of management has been maintained throughout; a seeds mixture including 1½ bushels of ryegrass, 10 lb. of cocksfoot and 1 lb. of wild white clover is sown at the end of April or early in May under a cover crop of oats. After the oats are harvested, the sward may be grazed in the autumn, and a seed crop is taken the following summer. The seed harvest nominally begins in the second week in July, but a week to ten days later is more usual. After harvest, the sward is used for grazing or hay, another seed crop being rarely taken. The harvested seed is sold to the wholesale merchant (or since 1946 to the Ministry of Agriculture) who bulks the seed. For the next cycle of seed production the farmer usually obtains seed from the bulked stocks of the seeds merchant. The usual generation time is thus two years, implying about 75 generations of multiplication under the same system of management between 1800 (when seed growing was already well established in Ayrshire), and the present day. Furthermore, the bulking of seed by the merchant means that there has been little opportunity for the development of local populations within the strain.

In Northern Ireland there are no absolute climatic limits to the growing season, and the present heading behaviour has been selected largely by the system of management of the seed crop. In the first place, any plant which produces heads during the summer of the seeding year will shed its seeds and die, or become greatly weakened and not be represented in the subsequent seed crop. Selection will thus operate for a sufficiently high winter requirement to prevent heading after an April sowing. However, the greater the number of fertile tillers per plant in the following spring the greater its representation in the seed crop, so an extremely high winter requirement will also be disadvantageous, and most plants will show a moderate requirement.

Secondly, in the first harvest year, selection will have been for types which give ripe seed at the date of cutting, about the middle of July, corresponding to ear emergence early in May. In Northern Ireland, with a lower spring temperature than Aberystwyth, ear emergence is not likely to occur much before the end of April, however low the critical photoperiod of the material. Consequently, while selection will have set an upper limit to the critical photoperiod, *i.e.* that value which will just give floral initiation in time for emergence at the beginning of May, there will be no such lower limit. This is borne out by the present tests for photoperiodic requirement, where nearly all the plants would initiate heads in an 11 hr. photoperiod, and 35 per cent. would do so even in a 9 hr. photoperiod.

As in Wimmera, it is apparent that more than 70 generations of selection have not produced strict uniformity in developmental

responses within the population, but that a wide range of winter requirements and photoperiodic responses are capable of producing a fairly uniform phenotypic result under the conditions for which the strain has been selected.

(iii) *Kent perennial ryegrass*

This is a late-flowering pasture strain derived from old pastures in the Romney Marsh and Wealden areas of Kent ; these have in many cases been under grass for several decades and are maintained under intensive grazing management, usually with sheep. Commercial seed is rarely produced directly from these old pastures, but the seed from the old pastures is used to sow down leys, which are themselves grazed intensively for five years before being harvested for seed (Hawkins, 1949). The Kent indigenous seed of commerce is thus two or more generations away from the old pasture. The traditional management varies slightly with the district, but in all cases consists of heavy grazing in the summer followed by light grazing in the winter. In all districts the field is grazed until the end of May before taking the seed crop.

As with Irish, there is little climatic limitation to the life-cycle, and the main selective action has been that of many years of intensive grazing. Under these conditions, only those plants will persist which can maintain themselves indefinitely under heavy grazing, and this implies a high proportion of vegetative to flowering shoots. Jenkin (1930) has shown that plants collected from old grazed pastures are prostrate in growth habit, and usually late-flowering, with little heading in the aftermath, and a high proportion of vegetative to fertile tillers. In the pasture strain, S.23, which is based on such material, Cooper (1951, 1957) has found this lateness of heading to be based on a high critical photoperiod ; similarly the low proportion of fertile tillers results from a high winter requirement and most of the tillers produced in mid to late spring fail to form heads in the same year.

Similar developmental responses are found in Kent ryegrass, the winter requirement for short-day or low temperature being greater than in Irish, and the photoperiodic requirement for floral initiation being higher, resulting in later heading. However, under grazing conditions, there has been no direct selection for uniformity of heading, and late or sparse heading has been a by-product of selection for the maximum production of vegetative tillers. One or two years of harvesting for seed would presumably select for a narrower range of heading dates and the variation in photoperiod response would be correspondingly reduced, particularly as in Kent ryegrass spring temperature no longer limits inflorescence development and the date of heading depends primarily on photoperiod. Seed multiplication would also favour a higher proportion of fertile tillers, and therefore a smaller winter requirement than in the original material.

The variation in date of ear emergence between stocks of Kent

ryegrass from different sources and different generations of multiplication has been studied by Hawkins (1950). He compared the original seed from permanent pastures with that from leys sown with this original seed, and also compared Wealden with Romney Marsh stocks and, as an additional check, with tiller plants collected from a Romney Marsh fattening pasture. The main character recorded was date of ear emergence in overwintered spaced plants. In 1946 and 1947 there were no obvious differences between the stocks from permanent pastures and those from once-grown stocks from leys, although the emergence date of the Wealden series was slightly earlier than that of the Romney Marsh series. In 1948, an early year, however, while all the once-grown stocks from leys were uniform and similar to the original Wealden, those harvested direct from Romney Marsh were more than ten days later than the others, and agreed in emergence date with the tiller plants from the permanent pasture. They also showed a much greater variance *within* stocks. Hawkins (1950) concludes that the grazing management of the leys, particularly grazing up to the end of May before taking a seed crop, is sufficient to maintain the high proportion of vegetative tillers and the lateness of heading of the original permanent pasture stocks.

Again, in Kent ryegrass as in Irish and Wimmera, although the strain is fairly uniform in heading behaviour in the field after overwintering, it is built up of a range of genotypes differing in exact winter requirement for short-day and/or low temperature, and also in the critical long photoperiod for floral initiation. Since heading is late, however, inflorescence development is not limited to any great extent by spring temperature and is under closer photoperiodic control than in Irish; the variation within the strain in response to photoperiod is thus less than in the early strains.

7. CONCLUSIONS

The present paper indicates how the range of heading responses found in each strain may have been selected by the combination of climate and management operating in each case. The three strains have evolved in rather different selective backgrounds. In Wimmera, the overriding factor is climatic, the limitation of the growing season by summer drought. This automatically selects for the annual habit, while self-regeneration from seed each year determines the optimum time of ear initiation and seed production in relation to the local distribution of temperature and photoperiod.

In Irish, there are no absolute climatic limitations to growth and that range of flowering responses has been selected which gives the maximum seed yield in the first harvest year under the usual system of management. Such selection largely precludes heading in the seed year, and therefore entails some degree of winter requirement. Since harvesting is comparatively early, the optimum time of heading is

limited by spring temperature, and a wide range of critical photoperiods is allowed.

In Kent, the primary selective effect is that of close and intensive grazing, both in the original permanent pasture and in the leys derived from it. Even when an infrequent seed crop is taken, the field is grazed till the end of May. This intensive grazing management selects for comparatively late heading, and also for a high proportion of vegetative tillers, *i.e.* a high winter requirement. It is not greatly altered by two years' harvest for seed.

In all strains, selection has operated to produce fairly uniform heading behaviour under the conditions of selection. This does not necessarily mean uniformity in developmental responses; in each strain a considerable range of genotypes differing in winter requirement for floral induction, and in critical photoperiod for floral initiation is allowed, provided these give a uniform end-product, *i.e.* date of ear emergence. This genetic variation between plants in developmental responses can often be revealed by altering the exposures to photoperiod and low temperature. A change in the environment may thus reveal genetic variation for selection to work on and produce a new population adapted to the new environment.

A similar range of genetic variation within phenotypically uniform local populations has been reported by Olmsted (1945) in *Bouteloua gracilis*, and by McMillan (1956*a, b*) in several other range grasses. While the flowering behaviour of each local population is closely adapted to local climatic conditions, considerable genetic variation is revealed when the population is grown at other climatic locations, or under artificially controlled photoperiods. Similarly, in the self-fertilising subterranean clover, Morley and Davern (1956) find that genotypes which flower at the same date at one station, need not necessarily do so in another environment.

In the present outbreeding strains of *Lolium*, genetic variation is also carried *within* plants in the heterozygous condition, and this serves to replenish the free variation between individuals in each generation. The extent of this potential genetic variation and its rate of release under selection will be discussed in a later paper.

8. SUMMARY

1. The aim of this work is a study of the genetic structure of three ryegrass populations, Wimmera, Irish and Kent, paying special attention to the amount of genetic variation carried within small groups of plants and its rate of release under selection. The main character studied is the time and extent of inflorescence development.

2. The present paper describes the genetic variation in heading behaviour between and within these populations after autumn and spring sowing in the field and examines the developmental responses to temperature and photoperiod responsible for these differences in field behaviour.

3. In overwintered material, the date of ear emergence is fairly uniform within each strain, Wimmera and Irish heading in early May, and Kent at the end of May. Year-to-year variation is greatest in the early strains, and can be attributed largely to spring temperature once the critical photoperiod has been reached. Most of the variation in heading date within each strain is genetic ; small differences may occur between stocks in both Irish and Kent ryegrass.

4. Spring sowing delays heading in all strains. In Wimmera, all seedlings produce heads even when sown in April, but with a greatly increased variance. In Irish and Kent, a progressive delay in sowing after January increases the proportion of non-heading plants, and after a critical sowing date (late March for Kent and mid-April for Irish) hardly any plants produce heads. There is a considerable range within each strain in the rapidity with which floral induction occurs after a spring sowing.

5. Greenhouse tests show that the annual Wimmera ryegrass has no obligatory "winter requirement" for short-day or low temperature before floral induction, but that heading is accelerated in many plants by either short-day or cold treatment. In Irish and Kent ryegrasses, few plants can produce heads without short-day or low temperature, the requirement for Kent being greater than for Irish. Considerable variation occurs within each strain in the response to short-day or low temperature.

6. The photoperiodic requirement for floral initiation also varies between and within strains. In Wimmera, all plants will initiate heads in a 9 hr. photoperiod, although ear emergence is delayed at this level ; in Irish, some plants will form heads in a 9 hr. photoperiod, but most require 11 hr. or more ; and in Kent, nearly all plants require more than 11 hr.

7. The range of developmental responses within each strain has been limited by the selective action of the environment. In Wimmera, the main factor is climatic, the restriction of the growing season by summer drought. In the perennial strains, management is more important, the limiting factors being many generations of seed growing in Irish ryegrass, and the effects of intensive grazing in Kent.

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