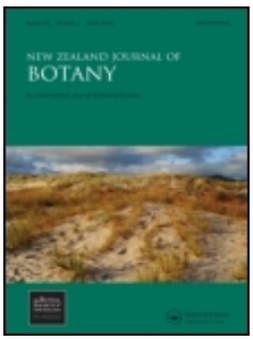

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GENETIC VARIABILITY IN COMMENCEMENT OF FLOWERING IN MEDICAGO LUPULINA L. IN THE SOUTH ISLAND OF NEW ZEALAND

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SUMMARY

Variability in date of commencement of flowering was investigated in *Medicago lupulina* L. from different localities in the South Island of New Zealand. The character was strongly inherited, and genetic differences between and within populations from different localities were demonstrated.

Although the species is classified as predominantly self-fertilising, the level of heterozygosity found suggests that outcrossing was not uncommon in natural populations.

INTRODUCTION

Medicago lupulina L. (black medick) is an annual, biennial, or perennial species, indigenous to temperate and subtropical regions of the Old World, and now widely distributed.

It is assumed that this species has entered New Zealand, mainly from Great Britain, several times since the beginning of permanent European settlement round about the middle of the nineteenth century. Seed entered as a constituent of general pasture mixtures, as pure seed for special-purpose pastures, and as an impurity in other seeds. However, it is most unlikely to have been cultivated in New Zealand during the last 40 years, and was cultivated only rarely before then (Hilgendorf, undated). There have probably been no deliberate importations since the introduction of seed certification in 1935. The species was first reported as naturalised in 1867 in the North Island (Hooker, 1864–67) and 1871 in the South Island (Armstrong, 1871), and was naturalised in many districts by 1899 (Kirk, 1899).

M. lupulina has a wide but discontinuous distribution in the South Island. It is mainly confined to habitats on stony, lime-containing soils,

especially along rocky beaches, rivers and roads, and railway verges, where it does not suffer severe competition from other species.

Great genetic variability is generally found in natural populations, not only of cross-fertilisers but also in predominantly self-fertilising plants, e.g., *Bromus mollis* (Knowles, 1943), *Geranium purpureum* (Baker, 1957), and *Avena fatua* (Imam and Allard, 1965).

Much geographic variation results from adaptation to local environments. Formation of ecotypes may be more common in self-fertilisers than in cross-fertilisers (Baker, 1953). *M. lupulina* is considered to be predominantly self-fertilised (Hollowell and Tysdal, 1948), and ecotypic differentiation may have taken place in this species since its establishment in the South Island.

The present paper describes a preliminary investigation of the genetic variability in date of commencement of flowering between and within populations of *M. lupulina* from different localities in the South Island.

MATERIAL AND METHODS

ORIGIN OF MATERIAL

Latitude, longitude, height above sea level, and mean annual rainfall for each of the six localities from which single plants were sampled for seed are listed in Table 1.

TABLE 1—Latitude, longitude, height above sea level, and mean annual rainfall for the different localities from which seed samples were collected

Locality	Latitude	Longitude	Approx. height above sea level (ft)	Mean annual rainfall (in)
Riwaka	41° 5′	172°58′	25	67
Rakautara	42°15′	173°48′	<4	40–45*
Kaikoura	42°25′	173°42′	<4	36
Conway Flat	42°39′	173°25′	90	36
Hakataramea	44°43′	170°30′	700	21
Cromwell	45° 2′	169°11′	720	18

* Estimated

The main collections were from Riwaka, Rakautara, and Cromwell with 26, 22, and 23 seed samples respectively, while Kaikoura, Conway Flat, and Hakataramea contributed 2, 2, and 6 seed samples respectively, a total of 81 seed samples.

Riwaka, Conway Flat, and Hakataramea collections were from stony, unstable river beds, subject to flooding. Rakautara and Kaikoura collections were from the stony verges of the beach just above the high-water mark, and the Cromwell collection from the road through the Cromwell Gorge. The greatest distance between plants sampled in each locality was not more than 100 yards.

The term "parent line" is used to refer to all plants derived from seed collected from each of the 81 plants sampled. A "progeny" comprises the plants obtained from open-pollinated seed of single plants selected from the parent lines.

The investigation was carried out in two stages.

PARENT LINES

In 1962 the 81 parent lines were planted as an incomplete lattice with nine blocks, each of nine parent lines. There were two replications and one row of 10 plants per plot. Commencement of flowering was recorded for each plant and analyses of variance were carried out for the mean and the range in number of days to first opened flowers of the parent lines. The range is the number of days between the first and last plants to start flowering. Its analysis of variance is reasonably valid (Kendall, 1946). Because a locality factor was not incorporated into the design, t-tests for comparing the locality means were only approximate. They were applied only to the large collections—Riwaka, Rakautara, and Cromwell.

PROGENIES

Progenies from four plants of each of nine parent lines were planted in 1963. Because of the large difference between the two replications of the parent trial, only plants of the early replication contributed to the progeny trial. The nine parent lines selected covered a wide distribution of mean and range in flower commencement, and the first and last plants of each line were always included. Four parent lines had large ranges compared with the other five lines. The design was a split-plot randomised block, with nine families (parent lines) as main treatments and the four progenies as sub-treatments. There were four replications and one row of 10 plants per plot. Separate analyses of variance were carried out for the mean and range within the two groups of families.

In both years seed was sown in boxes in autumn and the plants transferred to the experimental areas at Lincoln (latitude 43° 38′ S) in early spring. The germination rate was high and the plants established readily from transplanting. In both years the plants were spaced at three feet between and within rows.

RESULTS

The mean date of commencement of flowering of the earliest parent line, line 50 from Kaikoura, was 16 October.

MEAN NUMBER OF DAYS FROM 16 OCTOBER TO FIRST FLOWERS IN PARENT LINES

Analysis of variance showed highly significant differences between replications (average difference 8.5 days) and between parent lines (Table 2).

TABLE 2—Analysis of variance for the mean and the range in number of days to first flower in 81 lines of *M. lupulina*

	d.f.	Mean m.s.	Range m.s.
Replications	1	2928.7**	43.0
Blocks adjusted	16	42.1**	120.6*
Parent lines	80	344.7**	169.0**
Intra-block error	64	15.3	52.1
Randomised block error	80	20.7	65.8

* probability 0.05–0.01
** probability <0.01

There were highly significant differences within each of the localities Riwaka, Rakautara, Cromwell, and Hakataramea, as shown by t-tests. Highly significant differences were found between the means of Rakautara and Cromwell (11 days), Rakautara and Riwaka (21 days), and Cromwell and Riwaka (10 days).

The frequency distribution of mean number of days to first flower in arbitrary four-day periods for each population is given in Table 3. The Riwaka and Rakautara populations were less variable than the Cromwell population.

TABLE 3—Frequency distribution of mean number of days from 16 October* to first flower for 81 lines of *M. lupulina* from six localities

Mean (days)	Locality					
	Riwaka	Rakau-tara	Kaikoura	Conway Flat	Haka-taramea	Cromwell
0–4	—	—	1	—	—	—
4–8	—	—	—	—	—	—
8–12	—	2	—	—	2	—
12–16	—	3	1	—	—	1
16–20	—	5	—	—	2	2
20–24	—	4	—	—	—	4
24–28	—	6	—	—	—	1
28–32	—	—	—	1	2	3
32–36	3	—	—	—	—	1
36–40	9	—	—	—	—	5
40–44	4	—	—	1	—	1
44–48	2	2	—	—	—	2
48–52	5	—	—	—	—	2
52–56	3	—	—	—	—	1
Number of lines	26	22	2	2	6	23
Locality mean (days)	43	22	8	35	20	33

* Mean date of commencement of flowering of the earliest parent line
Least significant difference between lines at 5% level : 8 days
at 1% „ : 11 days

RANGE IN NUMBER OF DAYS TO FIRST FLOWER WITHIN PARENT LINES

Analysis of variance showed no differences between replications, and highly significant differences between parent lines (Table 2); t-tests indicated highly significant differences within each of the main localities and suggested highly significant differences between the means of ranges for Rakautara and Riwaka (8 days), and Cromwell and Riwaka (10 days), but not between Rakautara and Cromwell.

TABLE 4—Frequency distribution of within-line range in number of days to first flower for 81 lines of *M. lupulina* from six localities

Range (days)	Locality					
	Riwaka	Rakau-tara	Kaikoura	Conway Flat	Haka-taramea	Cromwell
0–4	1	—	—	—	—	—
4–8	8	1	—	—	—	1
8–12	8	3	—	—	2	4
12–16	5	3	—	—	1	3
16–20	2	5	—	1	2	2
20–24	1	6	—	—	1	3
24–28	1	2	—	—	—	3
28–32	—	1	1	—	—	4
32–36	—	1	—	—	—	2
36–40	—	—	—	—	—	1
40–44	—	—	1	—	—	—
44–48	—	—	—	—	—	—
48–52	—	—	—	1	—	—
Number of lines	26	22	2	2	6	23
Locality mean range (days)	11	19	36	33	15	21

Least significant difference between lines at 5% level : 15 days
at 1% „ : 20 days

There was no correlation between mean and range in number of days to first flowering. The range in number of days to first flower in arbitrary classes of four days showed wide frequency distributions (Table 4). Only parent lines with either a small (7–13 days) or a large range (26–39 days) were included in the progeny trial. One of the parent lines was from Riwaka, one from Kaikoura, and the other seven from Cromwell (Table 6).

PROGENIES OF FOUR PLANTS FROM EACH OF NINE SELECTED PARENT LINES

Analysis of variance for mean number of days to first flower showed very highly significant differences between families from parent lines,

TABLE 5—Analyses of variance for mean number of days to first flower for four progenies from each of five parent lines of *M. lupulina* with small range and four parent lines with large range

	Small range		Large range	
	d.f.	m.s.	d.f.	m.s.
Replications	3	3.7	3	7.1
Families from parent lines (1)	4	2021.5***	3	2705.5**
Error 1	12	4.4	9	4.2
Progenies within families from parent lines (2)	15	23.4***	12	260.5***
Error 2	45	2.2	36	3.7

** probability 0.01–0.001
*** „ < 0.001

TABLE 6—Mean for and range in number of days to first flower for five parent lines of *M. lupulina* with small range and four with large range in 1962, and mean for four progenies from each parent line and range in four progeny means in 1963

	1962—Parent Lines		1963—Progenies	
Parent line no.	Mean* (days)	Range** (days)	Mean* (days)	Range† (days)
SMALL RANGE				
7—Riwaka	51.2	10.1	52.2	1.5
61—Cromwell	51.1	11.3	56.0	4.0
70—Cromwell	54.8	12.0	54.2	9.4
72—Cromwell	20.1	6.6	31.3	1.6
77—Cromwell	18.6	12.9	36.8	6.1
			L.S.D.†† between family means	between progenies within families
	at 5% level		1.6	2.1
	at 1% level		2.1	2.8
	at 0.1% level		3.2	3.7
LARGE RANGE				
50—Kaikoura	2.9	30.2	17.5	7.0
62—Cromwell	38.6	39.3	46.3	36.6
66—Cromwell	38.3	32.4	40.1	19.4
74—Cromwell	43.6	25.7	42.5	16.0
			L.S.D.†† between family means	between progenies within families
	at 5% level		1.6	2.8
	at 1% level		2.4	3.7
	at 0.1% level		3.5	4.9

* Number of days from 16 October
** Number of days between first and last plants
† Number of days between means of first and last progenies
†† Least significant difference

and between plants within families, for both the small-range and the large-range groups, but no effect of replications (Table 5). Families from all parent lines, except line 7 (Riwaka) and line 72 (Cromwell), showed significant differences between progenies (Table 6).

There was a highly significant positive correlation in date of first flowering between the 36 individual parent plants in 1962 and the means of their progenies in 1963 ($r=0.96$). The regression equation is—

$$\text{Progeny} = 0.57 \text{ parent} + 29.7 \text{ days}$$

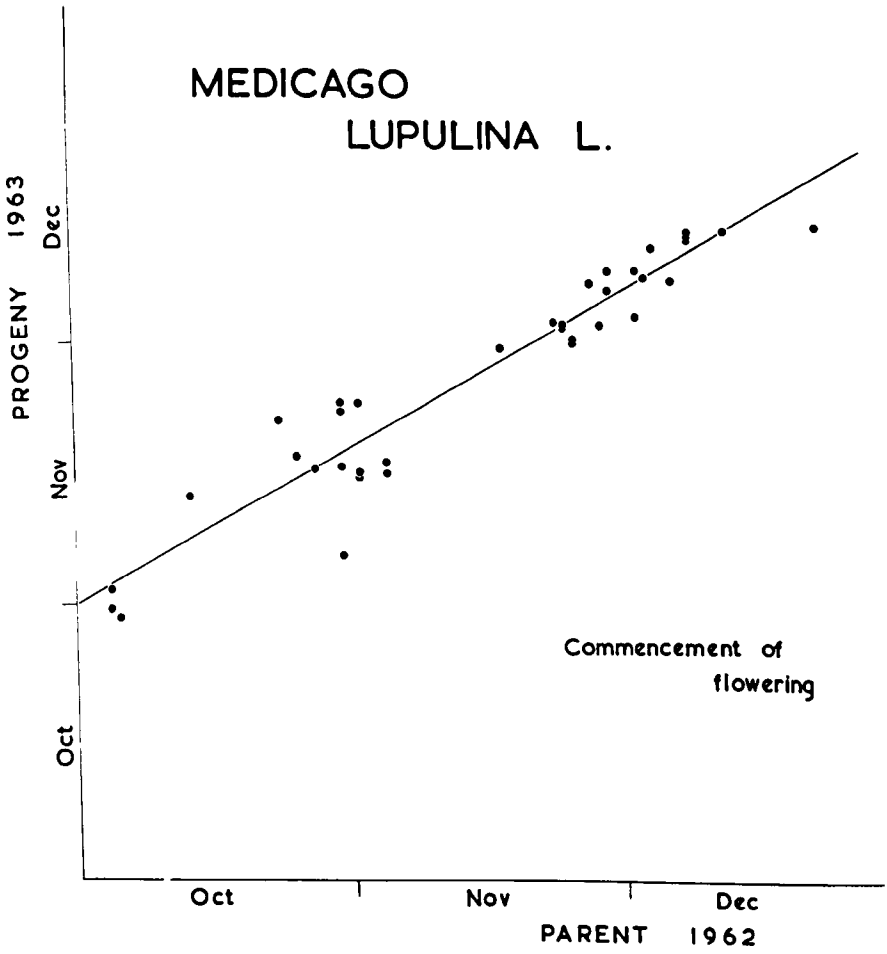

FIG. 1—Relationship in commencement of flowering between 36 parent plants of *M. lupulina* in 1962 and the means of their progenies in 1963. Regression equation: Progeny = 0.57 parent + 29.7 days.

DISCUSSION

The parent-progeny correlation confirmed that commencement of flowering was strongly inherited in *M. lupulina.* The expression of this character was clearly modified by environmental effects, which, in this investigation, appeared to be edaphic and seasonal.

There was a difference of eight and a half days between the replication means for mean commencement of flowering in the 1962 trials. Plants in the second replication were conspicuously less vigorous, and this could well have been responsible for the delay in flowering. These differences may have been caused by differences in soil reaction. Soil samples from the earlier vigorous replication had pH 5.5 and 5.7, and from the later replication, pH 4.8 and 4.9.

The difference between the parent lines in 1962 and their corresponding progenies in 1963, as shown by the regression equation, would be due mainly to a seasonal effect. Development was particularly slow during September 1963, when only 114 hours of bright sunshine were recorded, compared with 186 in September 1962.

There were genetic differences in flowering commencement between the locality means. Without more detailed study we can only speculate on the factors involved.

(*a*) *Different origins:* The population in each locality may have developed from distinctly different samples of the gene pool of the species. However, although the localities are discrete, there is no absolute barrier between them, as the seed of *M. lupulina* is readily disseminated by sheep, which are regularly moved over long distances in New Zealand. The age and stability of local populations is unknown and could be rather limited in some habitats, especially in river beds subject to periodic flooding.

(*b*) *Sampling technique:* From each population seed was collected once only. A comparison of different sampling dates throughout a year, and over a number of years, would be necessary to establish the importance of this factor.

(*c*) *Environmental adaptation:* Edaphic, climatic, and biotic factors limit the distribution of any species. *M. lupulina* appears to be restricted to lime-containing soils subject to periodic droughts, where competition pressure from other species is low. It is postulated that periodic droughts would be a strong selective factor in some localities. Under early and severe drought conditions, early-flowering genotypes would have an adaptive advantage. Under a more liberal moisture supply, late-flowering plants would have more vegetative growth before coming into flower and consequently would have a greater potential reproduction rate than early-flowering plants. This could explain the preponderance of early-flowering genotypes at Rakautara, where the water-holding capacity was extremely low and the fluctuating salty-water table restricted the rooting zone. Late-flowering lines would be more common in the river bed at

Riwaka, with its high rainfall and high water table. It could also explain why Cromwell, with its mosaic of contrasting habitats with large differences in aspect, depth of soil, and soil moisture supply, had the most heterogeneous population.

It was shown that out of nine selected parent lines, seven were heterogeneous for flowering time. The range within individual progenies suggests that in each locality a large proportion of plants were heterozygous for this character. The lower range at Riwaka may be the result of less outcrossing or of lower variance in this population.

No other measurements were taken, but each population was also extremely variable in other characters, such as habit, size and number of leaves, number of stems, and length of internodes, which are normally quantitatively inherited. However, amount of outcrossing can be measured only when a suitable monogenic difference is present.

M. lupulina sets as much seed when selfed as when open-pollinated, so insects or wind are not needed for fertilisation. Nevertheless, plants are visited regularly by honeybees and other pollinating insects. In its New Zealand habitats, *M. lupulina* flowers from early spring to autumn. Its breeding system could vary from complete self-fertilisation to considerable outcrossing, depending on the number of effective pollinating insects. A similar system was suggested for *Senecio vulgaris* (Haskell, 1953). Some genotypes may be less subject to outcrossing than others, and these could perhaps be recognised as biotypes, a situation also found in *Microseris douglasii* (Chambers, 1955).

This preliminary investigation suggests that *M. lupulina*, with its flexible breeding system, may have developed early- and late-flowering ecotypes in New Zealand. A more comprehensive sampling of chosen localities could show whether this has occurred.

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REFERENCES

ARMSTRONG, J. F. 1871: On the naturalized plants of the province of Canterbury. *Trans. Proc. N.Z. Inst. 4*: 284–91.

BAKER, H. G. 1953: Race formation and reproductive method in flowering plants. *Symp. Soc. exp. Biol. 7*: 114–45.

——— 1957: Genecological studies in *Geranium* (section Robertiana). General considerations and the races of *G. purpureum*. *New Phytol. 56*: 172–92.

CHAMBERS, K. L. 1955: A biosystematic study of the annual species of *Microseris*. *Contr. Dudley Herb. 4*: 207–312.

HASKELL, G. 1953: Adaptation and the breeding system in groundsel. *Genetica 26*: 468–84.

HILGENDORF, F. W. (undated): "Pasture Plants and Pastures of New Zealand." Sixth Edition, Whitcombe and Tombs, Christchurch.

HOLLOWELL, E. A. and TYSDAL, H. M. 1948: The need for seed is urgent. "Grass." *Yb. U.S. Dep. Agric.*: 343.

HOOKER, J. D. 1864–67: "Handbook of New Zealand Flora." Reeve and Co., London.

IMAM, A. G. and ALLARD, R. W. 1965: Population studies in predominantly self-pollinated species. VI. Genetic variability between and within natural populations of wild oats from differing habitats in California. *Genetics 51*: 49–62.

KENDALL, M. G. 1946: "The Advanced Theory of Statistics." Charles Griffin and Co., London.

KIRK, T. 1899: "The Students' Flora of New Zealand." Government Printer, Wellington.

KNOWLES, P. F. 1943: Improving an annual brome grass, *Bromus mollis* L., for range purposes. *J. Am. Soc. Agron. 35*: 584–94.