EVOLUTION IN SEGREGATING GENOTYPE MIXTURES OF SUBTERRANEAN CLOVER

P.G.H. Nichols[1] and P.S. Cocks

Plant Sciences and Centre for Legumes in Mediterranean Agriculture, University of Western Australia, Nedlands, Western Australia, 6907, Australia

[1]Current address: Agriculture Western Australia, Baron-Hay Court, South Perth, Western Australia, 6151, Australia

ABSTRACT

A complex segregating mixture of subterranean clover (*Trifolium subterraneum* L.), was sown in 1978 at Nabawa and Mt Barker, two contrasting sites in Western Australia. Seed harvested from both sites from 1980-1994 was grown out at Shenton Park in 1995, along with seed of the original mixture. Populations diverged markedly for mean days to first flowering (DFF) within three seasons. No further trends in mean DFF occurred in plants derived from the next 14 years, although standard deviation declined significantly in plants from Mt Barker. Between season differences in mean DFF were correlated with estimated growing season length at Nabawa and with May-October rainfall at Mt Barker.

KEYWORDS

Subterranean clover, *Trifolium subterraneum*, ecology, genotypes, mixtures, evolution

INTRODUCTION

Subterranean clover (*Trifolium subterraneum* L.), a winter growing annual pasture legume, is sown over 20 million ha. in southern Australia. Previous studies (Rossiter, 1977) have examined factors leading to the long-term success of subterranean clover genotypes in mixtures of genetically pure strains. However, segregating populations should provide a much greater array of genetic variation for natural selection to act upon, resulting in a much closer definition of characteristics important for long-term success.

This paper reports on the evolution over 18 yr of a segregating population of subterranean clover sown in two different environments in Western Australia. Time to first flowering was the character examined, as it was expected to reflect differences in mean growing season length of the two environments.

EXPERIMENTAL

A bulk F_2 mixture, derived from 253 crosses, was sown in 1978 at Mount Barker (350 km south of Perth) and Nabawa (470 km north of Perth) in Western Australia. Sowing rate was 25 kg ha^{-1} with plots 0.5 ha. Trials were cropped with wheat during 1991 and 1994. Otherwise, they were grazed by sheep. Seed samples were collected annually from both sites, except 1991, by digging 20 random quadrats (1 m x 0.25 m) to 50 mm depth. Seeds were threshed from burrs, placed in sealed foil envelopes and maintained at 4° C. Residual seed of the original sown mixture was also maintained. When tested in 1993, seeds from 1978 and 1979 were not viable and could not be used in the experiment.

Scarified seeds from each population were inoculated with Group C rhizobia and sown in a glasshouse at the University of Western Australia Field Station, Shenton Park on 15 May, 1995. Fifty four random seedlings of each population were transplanted 25 cm apart to the field, 43 d after sowing, into a loam-dressed sand at Shenton Park. Plants were irrigated to prevent moisture stress and hand weeded. Fertiliser (100 kg ha^{-1} of 3:1 super potash) was applied on 30 May and 15 September.

Flowering time was measured as the number of days from sowing to appearance of the first flower. Plants were checked every 3-4 days. A soil moisture balance model, using weekly rainfall recordings and long-term mean weekly evaporation (Epan) levels, was constructed for Nabawa and Mt Barker to enable estimation of growing season length. A maximum soil water holding capacity of 75 mm was assumed at both sites. Weekly soil moisture balance was calculated as the cumulative difference between rainfall input and an indexed evaporative loss of 1.21 x $Epan^{0.75}$ (Prescott,1934). Statistical analyses were conducted using SYSTAT for Windows, Version 5.

RESULTS

From 1978-1994, mean annual rainfall at Nabawa was 432.3 mm, compared to 635.9 mm at Mt Barker. The calculated mean growing season lengths were 19.4 wk at Nabawa and 31.3 wk at Mt Barker, which closely match anecdotal records.

Figure 1 shows the mean days to first flowering (DFF) at Shenton Park of the original mixture and for plants derived from Nabawa and Mt Barker seed collected from 1980-1994. It is apparent that the populations from the two sites diverged both markedly and rapidly in mean DFF. After just three seasons, mean DFF of plants from the two sites differed significantly from both the original mixture ($P < 0.001$) and from each other ($P < 0.001$).

From 1980-1994 no significant trend occurred in mean DFF of plants derived from either site. However, some significant variations in mean DFF occurred from year to year. For the Mt Barker population, plants from 1993 seed differed significantly in mean DFF from both 1992 ($P < 0.01$) and 1994 ($P < 0.001$), while plants from 1989 and 1990 seed also differed significantly ($P < 0.05$). Similarly, for the Nabawa population, plants from 1981 seed differed significantly in mean DFF to those from both 1980 ($P < 0.001$) and 1982 ($P < 0.001$), and those from 1987 seed also differed significantly from 1988 ($P < 0.01$). For the Nabawa population, the between-season differences in mean DFF could be partially explained by the significant correlation ($r^2 = 0.33$, $P < 0.05$) with growing season length. There was no such correlation at Mt Barker. However, in this case, a significant correlation ($r^2 = 0.31$, $P < 0.05$) was found between mean DFF and May-October rainfall.

Standard Deviation for DFF was much greater in plants derived from Mt Barker seed than from Nabawa throughout the trial period (Figure 2). There was a significant negative trend in Standard Deviation of DFF between plants from the original mixture and plants derived from the 1994 populations from both Mt Barker ($P < 0.01$) and Nabawa ($P < 0.05$). For the Mt Barker population, a significant ($P < 0.05$) but smaller negative trend continued from 1980-1994. There was no such trend for the Nabawa population.

DISCUSSION

This experiment clearly demonstrates the effects of natural selection on mixed genotype populations. From the one highly variable population, two quite different populations, in terms of flowering time, evolved at the two experimental sites. Furthermore, most of this evolution occurred within just three seasons. Had it been possible to use seed from the first two seasons, it is likely that even more rapid divergence would have been observed.

These results confirm the importance of appropriate flowering time for adaption of subterranean clover to particular environments. At Nabawa, with a short growing season, there was very strong selection pressure for early flowering genotypes. This is readily explained by the need for flowering to start early enough for adequate seed to be produced before the onset of summer drought.

In the longer growing season environment of Mt Barker, genotypes with midseason flowering were favoured, although the wider range of DFF indicates that selection pressure for optimum DFF was not as strong as at Nabawa. Even though mean DFF did not change further over time, variability continued to decline at Mt Barker, suggesting that even after 17 seasons, selection was still occurring against both earlier and later flowering genotypes. Late flowering genotypes would have been at a selective disadvantage, as they require an even longer growing season for maximum seed production. Early flowering genotypes were also at a competitive disadvantage. Possible explanations include their lower seed production capacity or the likelihood that continued rain in spring following plant senescence leads to significant seed spoilage.

These results have significance for the breeding of annual pasture legumes. Genotypes with an appropriate DFF for a particular target environment can be readily selected after just three seasons from a segregating population, by allowing them to regenerate in that environment under natural selection.

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REFERENCES

Prescott, J.A. 1934. Single value climatic factors. Trans. R. Soc. S. Aust. **58**: 48-61.

Rossiter, R.C. 1977. What determines the success of subterranean clover strains in south-western Australia? In 'Exotic species in Australia - their Establishment and Success'. Proc. Ecol. Soc. Aust. **10**: 76-88.

Figure 1

Mean DFF at Shenton Park of plants derived from an original segregating mixture sown in 1978 at Nabawa and Mt Barker and from seed collected from 1980-1994 at those sites.

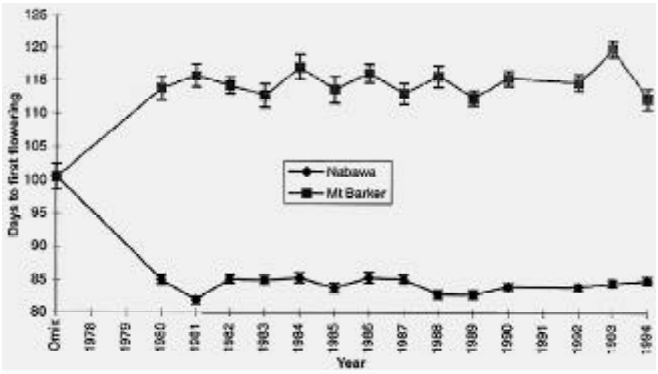

Figure 2

Standard deviation for DFF at Shenton Park of plants derived from an original segregating mixture sown in 1978 at Nabawa and Mt Barker and from seed collected from 1980-1994 at those sites.