

GENETIC VARIATION IN TRIFOLIUM: COMPARISON OF SOME OUTCROSSING AND INBREEDING SPECIES FROM TURKEY

S.J. Bennett

Co-operative Research Centre for Legumes in Mediterranean Agriculture, University of Western Australia, Nedlands, WA 6907.

Abstract

A collection in S.W. Turkey in June 1996 provided the material to study the genetic variation found in five species of *Trifolium*; two outcrossing and three inbreeding. The material was grown as spaced rows and a number of morphological characters were scored. The genetic variation found in three characters; days to flowering, petiole length and leaflet length is discussed in this paper and related to some of the ecogeographic variables that were recorded at the site of collection. The outcrossing species were found to contain a greater amount of genetic variation within and between sites than the inbreeding species, and the variation was found to be correlated to latitude, and altitude. The species with a wide natural distribution; *T. tomentosum* and *T. resupinatum* contained more variation than those species with a narrow natural distribution. Variation in the inbreeding species, *T. glomeratum* and *T. tomentosum* could not be attributed to edaphic variation.

Key words: Trifolium, Turkey, inbreeding, outcrossing, genetic variation.

Many annual pasture species that have been commercialised for agriculture are inbreeding, such as subterranean clover (*T. subterraneum*). These species are easy to maintain as distinct cultivars as they contain a higher degree of homozygosity within a cultivar and therefore little variation. However, there is currently a drive in southern Australia to diversify the pasture species available and this may involve the development of outcrossing cultivars, as well as the more traditional inbreeding ones.

There are a number of advantages of using outcrossing species compared to inbreeding species. They have the potential to adapt faster to a new environment as a result of the heterozygosity contained within the population, and there are increased levels of gene flow occurring between populations, compared to inbreeding species. This in turn maintains a greater degree of genetic variation within a population. Recent studies have shown that the amount of variation contained within inbreeding species is greater than had previously been assumed (3, 7), with quantifiable adaptation occurring over a range of environments. The question that still remains though, is how rapid is that adaptation and are there any ecogeographical factors driving the adaptation? Also, are there any other factors that are important in determining the amount of variation present within a species, other than breeding system?

A collection in Turkey in 1996 (4) provided the material that would give a preliminary indication to some of these questions and the basis on which to plan further studies. The aim of the experiment was to determine the amount of genetic variation that was present in five species of *Trifolium* collected in SW Turkey, and to relate that variation to distribution of collections, plus a number of ecogeographical factors. Preliminary results are discussed in this paper.

Materials and methods

Seed was collected from S.W. Turkey in June and July 1996 as part of a co-operative collecting mission between the Centre for Legumes in Mediterranean Agriculture (CLIMA), Australia and the Aegean Agricultural Research Institute (AARI), Turkey (4). Five species were chosen for further evaluation; two outcrossing (*Trifolium michelianum* Savi and *T. resupinatum* L.) and three inbreeding species (*T. clypeatum* L., *T. glomeratum* L. and *T. tomentosum* L.) and these were collected at a total of 39 sites. However all five species were not collected at any one site. A full set of passport data accompanied each collection and this will be used in later analyses.

The material was grown at Medina Research Station, WA as part of the Trifolium Genetic Resources Unit evaluation and was planted according to Snowball (8). A total of four collection sites of *T. clypeatum* were included, 14 *T. glomeratum*, 26 *T. tomentosum*, 15 *T. resupinatum* and 5 *T. michelianum*. Between five to ten plants were grown from each collection site and each collection was planted as far as possible from any other collections of the same species.

A number of morphological characters were measured, and these will be evaluated in conjunction with the passport data that is available for each site. The author is aware that because different combinations of species were collected at each site, for many univariate measures, the data cannot be analysed as a whole and as such will be broken down into a number of different relationships. Also within the scope of this paper it is only possible to describe a limited number of the results and therefore further analysis will be published in a later paper. The characters analysed in this paper were time of flowering from date of sowing, leaflet length and petiole length. The data were analysed using the statistical computer package, SPSS for Windows v.7.5.1.

Results

The mean, standard error of the mean and the variance of each of the three characters listed above are shown in Table 1 for each species. This shows that *T. clypeatum* flowers on average 11 days earlier than any of the other species, although there is a relatively large percentage coefficient of variance (% CV). The latest flowering species is *T. glomeratum* at 170 days, 12 days later than any other species, and with a lowest %CV. *T. tomentosum* and *T. resupinatum* both flowered at about the same time, with *T. resupinatum* showing a greater variation in flowering time. Leaflet length (Lft_len) was found to correspond to the size of the plant, with *T. clypeatum* and *T. michelianum* having the largest leaflets, and *T. tomentosum* the smallest. Petiole length was found to be independent of the growth characters and was therefore a good taxonomic character to differentiate between the species. *T. michelianum* and *T. tomentosum* both had long petioles, with *T. tomentosum* showing a very large variation for this character. The other three species had petiole lengths less than half of those scored for *T. tomentosum* and *T. michelianum*.

There are some interesting correlations between the morphological characters and some of the ecogeographical data collected at each site, calculated as site means per species. The two outcrossing species, *T. michelianum* and *T. resupinatum* show more correlations to their ecogeographical environment than the inbreeding species, with *T. michelianum* showing good correlations between the date of flowering and latitude ($R^2=0.899^{**}$, $P^{**}<0.01$) and altitude ($R^2=0.719^*$, $P^*<0.05$), and leaflet length and latitude ($R^2=0.832^{**}$). Flowering date in *T. resupinatum* was correlated to altitude ($R^2=-0.514^{**}$). The inbreeding species, possibly as a result of less variation being present in the characters scored, show few correlations with their ecogeographic environment. The only correlations above 0.5 are found in *T. clypeatum* between leaflet length and latitude ($R^2=0.937^{**}$) and pH ($R^2=0.920^{**}$).

As a result of different combinations of species being present at each site, it was decided that the analysis of variance would be computed on a number of subsets of the data. The chosen subsets were: a) two sites (12 and 18) where four species were present (Group A = *T. glomeratum*, *T. tomentosum*, *T. michelianum* and *T. resupinatum*) and b) 11 sites where *T. tomentosum* and *T. resupinatum* were present (Group B), which would give a comparison between an inbreeding and outcrossing species, where both species were widely distributed.

Within these two groups the data were tested for normality and for homogeneity of variances using Levene's test. All of the characters scored were found to show a normal distribution, but days to flowering and leaflet length in both groups showed heterogeneity of variances. For this reason the data were transformed using a logarithmic transformation, as this reduces the heterogeneity present in the two groups (1). Analysis of variance was then computed on the transformed data.

In group A, days to flowering were found to be significantly different between the four species, and there were significant differences between the two sites, however there was no interaction between the two. This was due to each species flowering later at site 12 which was at a higher latitude, but the flowering

times remained in the order shown in Table 1. Both leaflet length and petiole length showed significant differences between species and sites, with an interaction also present between species and sites. The interaction in leaflet length was found to be due to the length decreasing with latitude in both the outcrossing species, but no change was present in the two inbreeding species. Petiole length was found to be constant between sites in *T. glomeratum* and *T. resupinatum*, but to increase with latitude in *T. tomentosum* and dramatically decrease in *T. michelianum*.

Table 1: Mean, standard error, and variance for selected characters by species over all collection sites.

In group B, days to flowering was significantly different between sites, and between sites within species, however there was no significant difference between the *T. tomentosum* and *T. resupinatum*. Fig. 1 shows the days to flowering of the two species in relation to latitude where it can be seen that there is little variation in *T. tomentosum* compared to *T. resupinatum* which is earlier at lower latitudes and later at higher latitudes. Both leaflet length and petiole length showed significant differences between species, sites and between sites within species. Leaflet length was similar in both species at lower latitudes, but as the latitude increased, the length of the leaflet increased in *T. resupinatum* and decreased in *T. tomentosum*. In comparison, petiole length was longer in *T. tomentosum*, and showed much greater variation at each site.

Fig. 2 shows the differentiation of the five species using only two characters. As only three characters have been discussed in this paper, there is no benefit to using any multivariate methods of discrimination on the character data alone. However, since Fig. 2 shows some differentiation between the species, the potential of using multivariate methods of analysis when all the morphological characters are included is great and this will be demonstrated in a subsequent paper.

Discussion

The preliminary results described above have shown the amount of variation that is present in the five species of *Trifolium* in just three characters. As expected the two outcrossing species; *T. michelianum* and *T. resupinatum* showed significant amounts of variation between and within sites and are correlated to some of the ecogeographical factors, particularly latitude and altitude, in the case of *T. michelianum*, and altitude, in the case of *T. resupinatum*. Beale et al. (2) found that *T. resupinatum* showed a preference for higher rainfall sites, and these tend to be at higher altitudes. *T. resupinatum* was the species that was collected from the greatest range of sites, and over the widest area. The large genetic variation that is contained within the population is therefore a reflection of its adaptation to a wide range of environments. It has previously been shown that outcrossing species contain greater within species variation than inbreeding species (6) due to the continual movement of genes between populations. It is for this reason that an environmental cline develops as gene flow is greatest between neighbouring populations.

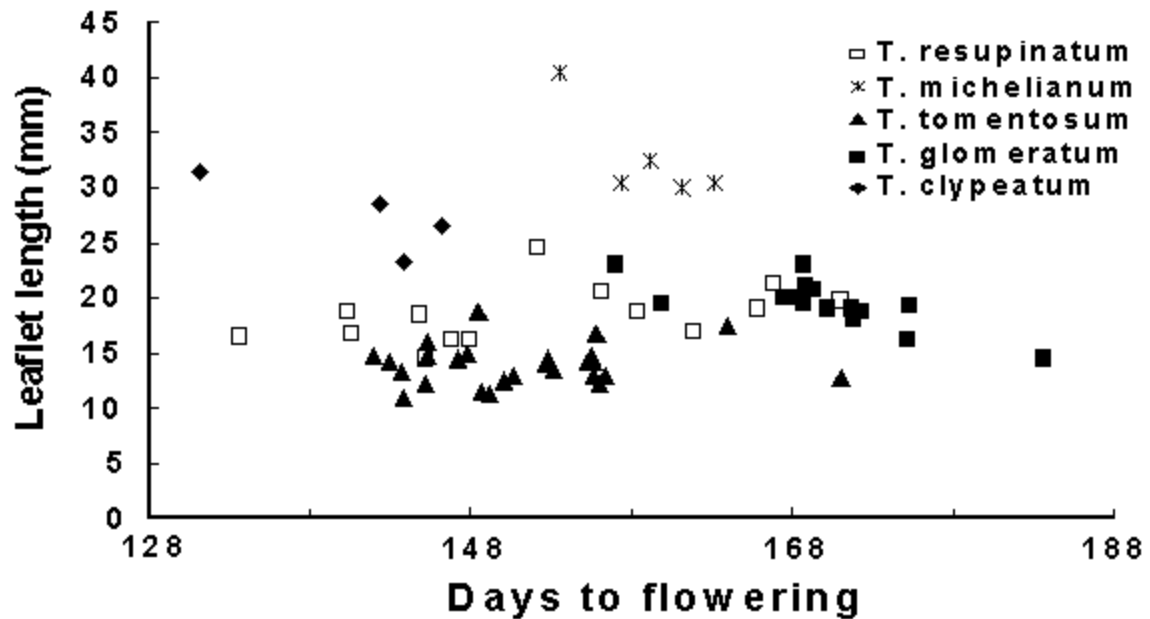


Figure 2

T. tomentosum was also collected from a wide range of environments, and at many of the same sites as *T. resupinatum*. This species also displayed a wide genetic variation, despite having an inbreeding reproduction system, although the variation was not correlated to any ecogeographic factors. The other two inbreeding species; *T. glomeratum* and *T. clypeatum* also showed no correlation to ecogeographic factors. Recent studies have shown that inbreeding species contain greater within population variation than had been generally accepted (3, 7) due to limited amounts of outcrossing occurring in the population and the movement of seed between populations by sheep and other animals. This transfer of genes is important in the adaptation of a population to an ever changing environment, but does not result in the environmental clines present in the outcrossing species. Consequently, the speed of adaptation to a new environment is slower in an inbreeding species.

The geographic or edaphic range also appears to be important in explaining the amount of variation present within a species. Both *T. resupinatum* and *T. tomentosum* were collected from a wide range of geographic and edaphic habitats and these two species exhibited the greatest variation between collecting sites. *T. glomeratum* and *T. clypeatum* were collected from much narrower distributions; geographically in *T. glomeratum* and edaphically in *T. clypeatum*, and these were the two species with the least amount of within and between collecting site variation.

Conclusion

The two outcrossing species have been shown to contain significant amounts of variation, both between and within collecting sites, and between site variation has been correlated to a number of ecogeographic factors. The inbreeding species have also been shown to contain large amounts of genetic variation between and within collecting sites, particularly in *T. tomentosum* which has a wide distribution in southern Turkey. Less variation is present in the species with a narrow geographic or edaphic range.

The preliminary results described in this paper suggest important implications in the drive for new pasture species in southern Australia. It has been recognised that it is important to increase the diversity of our pastures (5) and so it is suggested that outcrossing species should be included in any evaluation trials, as they may show a faster adaptation to the environments in which they are targeted.

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