ELECTROPHORETIC ANALYSIS OF A SINGLE LOCUS OF THAMNOCHORTUS INSIGNIS Mast.

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Honours project 1992

ABSTRACT

Isozyme electrophoresis of a single polymorphic locus, Shikimic Acid Dehydrogenase (SDH), of *Thamnochortus insignis* Mast. (Restionaceae) is presented. Five populations from the Southern Cape Province were used in the analysis. A conservative and liberal estimate of allele diversity was carried out as a result of difficulties encountered when interpreting the banding patterns on the gels. Significant deviations from Hardy-Weinberg equilibrium, contrary to predictions made for a dioecious wind pollinated species, were found for the populations at De Hoop, Klipfontein and Witbakenkop. A liberal estimate of the allelic diversity among the populations produced a F_{ST} of 0.242 which implies that very little gene flow occurs between the populations at Springfield and De Hoop, and between both of these and the populations at Klipfontein, Hectorskraal and Witbakenkop. Isolation by distance and different times of flowering are suggested as possible barriers to gene flow. A conservative estimate of the allelic diversity among the populations produced a F_{ST} of 0.038 which implies high levels of gene flow among populations, as is predicted for dioecious wind pollinated species. The allelic diversity within populations is high overall (F_{IS}=0.317), implying that the genetic neighbourhoods are relatively small with reduced gene flow within populations. This evidence is contrary to that predicted from a direct estimate of gene flow for another species of Restionaceae.

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INTRODUCTION

The genetic structure of populations results from the joint action of mutation, selection, and drift which in turn operate within the historical and biological context of each plant species (Loveless and Hamrick 1984). By definition genetic structure refers to the nonrandom distribution of alleles or genotypes and in the current study, this is summarised for *Thamnochortus insignis*, using a single locus, namely that of Shikimic Acid Dehydrogenase (SDH). The fact that an isozyme is being used to investigate the genetic structure, somewhat changes the relevance of the opening statement. In the context of this study selection may therefore be ignored. Instead mutation and more importantly genetic drift and migration play the leading roles in producing the genetic structure of the species under investigation, bearing in mind that interpretation is limited by the use of just a single locus. Naturally, knowledge of the genetic structure based on many loci produces a more accurate picture (Stebbins 1989).

Thamnochortus insignis is a member of the Restionaceae, a family of dioecious, wind pollinated plants. The species is distributed along the southern coast of the Cape Province, its range is relatively narrow, and restricted to the coastal plain stretching from Bredasdorp in the west to the Gouritz river in the east.

Plant breeding systems as a whole have been identified as a major factor influencing genetic structure (Loveless and Hamrick 1984). Obligate outcrossing as in this species would be expected to increase genetic neighbourhoods and reduce population subdivision and incompatibility mechanisms could cause pollen dispersal and genetic neighbourhoods to become larger. On the other hand, assortative mating and unequal sex ratios can reduce genetic neighbourhoods and generate differentiation of a population. Wind pollination is generally associated with low genetic variation among populations with large genetic neighbourhoods within populations (Loveless and Hamrick 1984). Honig *et al.* (1992), in a study of the efficacy of pollen movement in populations of *Staberoha banksii* (Restionaceae), concluded that wind was an efficient vector of pollen transfer and no leptokurtic distribution of pollen

densities away from plants were found within populations. They concluded that genetic neighbourhoods were large, so that differentiation within a population would be kept to a minimum.

Factors which may reduce the gene flow from population to population over a number of generations may include a patchy distribution of populations with isolated outliers, or shifts in phenology. No information regarding the phenology of the populations of *T. insignis* is available and so interpopulational distances are, currently, the only available measure of genetic isolation.

Theoretical studies have shown that only a small amount of long distance gene flow is needed to prevent population differentiation for neutral alleles (Loveless and Hamrick 1984). However, competing theories regarding gene flow as a potentially constraining or creative force, suggest that under different demographic structures either one of these forces is acting (Slatkin 1987). If the geographic distribution of a species remains the same and if local populations persist for long times, as would be the case for *Thamnochortus insignis*, then gene flow occurs primarily through the movement of individuals between established populations, with the amount of gene flow dependent on the breeding biology of the species. Presumably, gene flow would be relatively high for this species. In such species gene flow is said to play a conservative role because it prevents the genetic differentiation of local populations and inhibits speciation (Slatkin 1987). Indirect methods of estimating gene flow (i.e. allele frequencies), however, do not themselves reveal how much gene flow is occurring (Slatkin 1987). Differences between direct methods (i.e. mobility of seed or pollen) and indirect estimates of gene flow indicate that movements of seed and pollen between existing populations cannot account for their genetic similarity. Slatkin (1987) suggests that the current patterns are probably due to substantial gene flow in the past.

Hamrick and Godt (1990) have collated evidence on the genetic structure for a wide variety of species. They found that for outcrossing wind pollinated plants the proportion of the total genetic diversity distributed among populations was low, with relatively high genetic diversity within populations, and with low total

genetic diversity compared to species with other breeding systems. On the other hand species with narrow geographical ranges have a relatively higher proportion of the total diversity among populations and so do monocotyledons in general. However, these patterns may not be sufficient to cause deviations in genetic structure from those predicted for a normal outcrossing wind pollinated species such as *T. insignis*.

Study sites:

The natural distribution of *Thamnochortus insignis* is from near Bredasdorp in the west to the Gouritz river in the east. Five populations were sampled along this range at Springfield, De Hoop Nature Reserve, Klipfontein, Hectorskraal and Witbakenkop (figure 1). Population structure at these sites varied, as did landscape and edaphic features.

Site descriptions:

The *Springfield* (1) population (34°40'35"S 19°55'20"E) was situated adjacent to a vlei and consisted of sparsely distributed, somewhat untidy looking individuals. The tussocks were small (<1m), perhaps due to young age. The site was level with a shallow sand layer overlying limestone. Average soil pH of 6.9.

The population at *De Hoop Nature Reserve* (2) (34°28'10"S 20°26'50"E) consisted of seedlings of no higher than 10cm above the ground. The site was level with deep sand. Individuals of the stand adjacent to the sampling area were relatively large individuals. Average soil pH of 7.9.

The population at the *Klipfontein* (3) site (34°16'10"S 21°13'15"E) was situated on a flat valley floor, with deep sands, between limestone ridges. The density of individuals was high with many seedlings inbetween. Average soil pH of 6.22.

The *Hectorskraal* (4) population (34°15'10"S 21°26'35"E) comprised a dense stand in a flat dune valley. The sand was light brown in colour. Average soil pH of 5.7.

The *Witbakenkop* (5) population (34°18'12"S 21°29'20"E) consisted of very large individuals (>2.0m) on deep level sands. Average soil pH of 5.7.

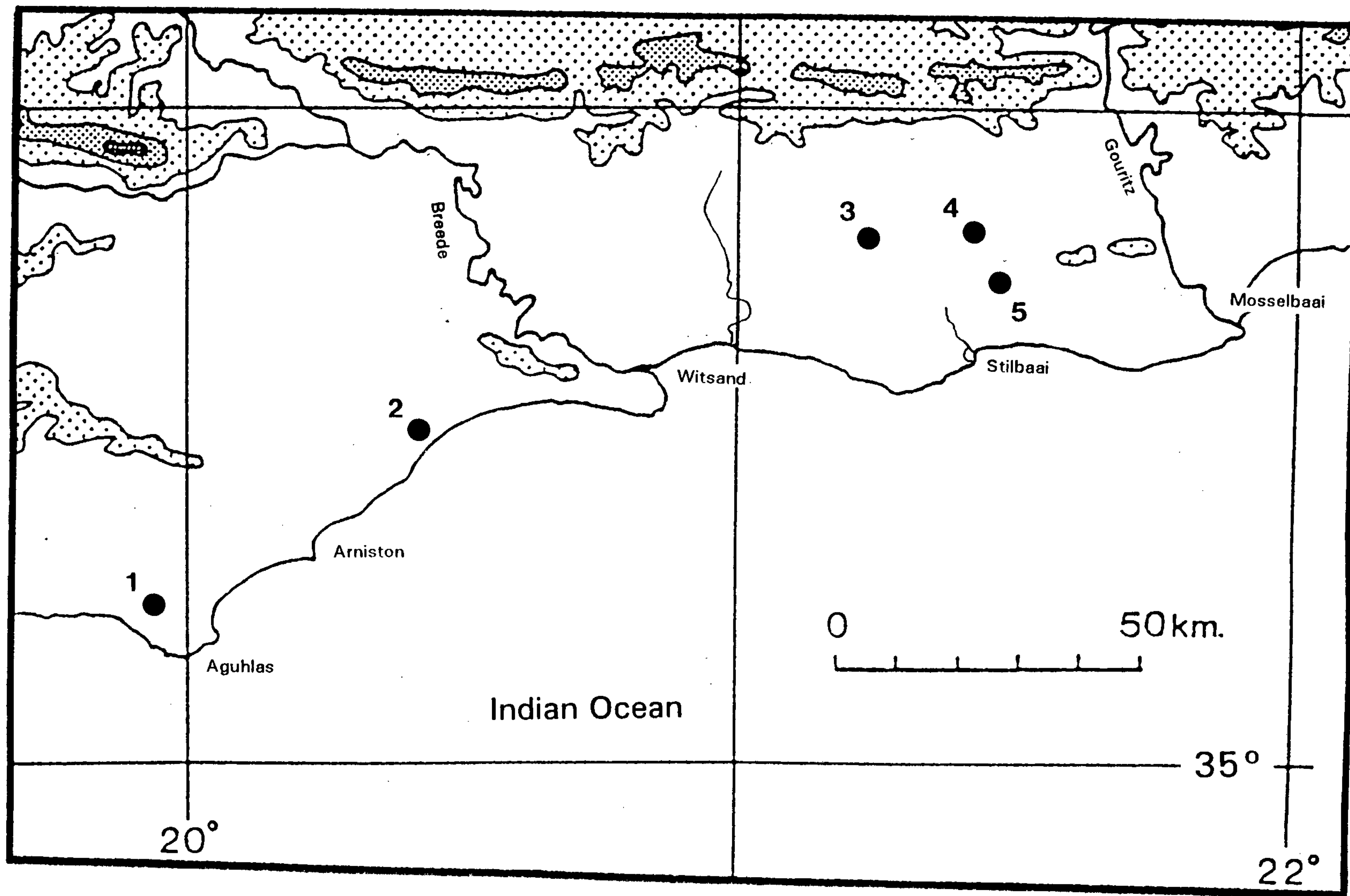


Figure 1: A map of the Southern Cape coast showing the localities of the five populations which were sampled. 1 (Springfield), 2 (De Hoop Nature Reserve), 3 (Klipfontein), 4 (Hectorskraal) and 5 (Witbakenkop).

MATERIALS AND METHODS

Sampling methods:

Forty individuals from each population were sampled. At most of the sites, seedlings were abundant, ranging from 3cm to 20cm high. Where seedlings were abundant, sampling was carried out along a transect with every second individual being collected and placed in a plastic zipper bag. The plastic bags were immediately placed in a cooler box containing dry ice. On arriving back at the laboratory specimens were immediately placed in a freezer at a temperature of between -10 and -20°C).

Enzyme extraction:

The enzymes were extracted using the grinding buffer described in table 1 (Cheliak and Pitel 1984). The ingredients were dissolved in distilled water, and mixed thoroughly using a stirrer. The pH was adjusted to 7.5. 0.025ml (1%) 2-mercaptoethanol (the vol. of dropper) was then added and the solution was made up to 25ml. with distilled water and stored in a 1°C fridge. For each specimen small amounts of material were ground in this buffer using a mortar and pestle. A few wicks were soaked in each grindate and placed in small plastic tubes. The remainder of the grindate was placed in separate plastic tubes. The plastic tubes were then placed in the deep freezer (-10 to -20°C). Wicks were cut out of filter paper to have dimensions of about 3mm.x 7mm.

Table 1: The grinding buffer used in this study (after Cheliak and Pitel 1984)

Dist.water	19ml
0.2M sodium tetraborate	1.91g
0.2M sodium metabisulphite	0.095g
0.25M ascorbic acid	1.24g
0.026M diethyldithiocarbamic acid	0.113g
0.1M maleic acid	0.29g
0.1M tris	0.3g
4% PVP	1g
bovine serum albumin *	7.6mg
NAD	5.13mg
NADP	4.25mg

* Bovine serum albumin helps in the extraction of the enzymes (Wendel and Weeden 1989)

Electrophoretic procedure:

In order to carry out or "run" electrophoresis specific gel buffers and corresponding electrode buffers need to be made up. For the purpose of this study, buffers for Shikimic Acid Dehydogenase (SDH) were made up. Table 2 lists the ingredients of the gel and electrode buffer used (i.e. Tris-EDTA Borate buffer). In order to make the gel 16.5ml of the buffer was diluted with distilled water up to 250ml. 31.25g of hydrolysed starch was then mixed thoroughly with approximately 60ml of this solution. The remainder was heated until it boiled, at which point it was mixed with the starch/buffer solution. This was then placed back over the heat until it boiled, after which the air was sucked out of the ehrlinmeyer flask and also out of the boiling starch solution until only large bubbles were seen to pass up through the hot starch solution. The solution was then poured into a shallow glass tray. Any small bubbles remaining in the gel were removed using a pasteur pipette, and the gel was then left to cool down a little, at which time, it was covered with cling wrap, and finally placed in the refrigerater at 1°C.

Table 2: Gel and electrode buffer recipe

Tris	21.8g
EDTA	1.52g
Borate	6.18g
Distilled water mixed with ingredients and made upto 1000ml	
Add HCL to achieve pH 8.0	

After half an hour the gel was retrieved from the cold room and the wicks holding the extracted enzymes were inserted in it. This was done by cutting the gel right through its thickness, lengthwise, about 1/4 of the way along it's width, producing a slit in which wicks were placed. Wicks were spaced evenly, and separated by a distance of about 3mm or more. Excess grindate on the wicks was taken off before they were placed in the gel. The cling wrap was replaced over the gel, and this was then taken back to the refrigerater where the electrophoretic run was carried out.

Up to thirty wicks were placed on the gels. Some gels consisted of individuals from one population only, while others had individuals from all five populations represented.

The electrode buffer (=gel buffer) was used at full strength and was poured into two elongated trays (4cm high). Buffer soaked sponges were used to connect the cathode, the gel and the anode permitting production of a current across the gel. Mean amperage for the runs was 50mA and mean voltage was 160V. After about 20 minutes the wicks were removed.

During the process of placing the wicks into the gel two wicks, soaked with bromothymol blue, were placed on either side of the gel. These acted as markers for the enzyme front. The gels were run for a period of 5 to 6 hours, and sometimes 7 hours, depending on the variable voltage setting and progress of the enzyme front markers.

Staining procedure:

The SDH enzyme was stained using the stain outlined in table 3. On completion of the gel run, the gel tray was removed from the electrodes and the gel removed from the tray, and placed in the staining dish containing the stain. Phenozene methyl sulphate (PMS) and MTT (Cheliak and Pitel 1984) was then put into the staining tray. This is because these chemicals break down when exposed to light and must therefore receive minimum amounts before the gel is incubated.

The gel was incubated in an oven at 37°C for 30 min., after which the oven was turned off and the tray was left in dark overnight.

Table 3: The staining recipe for Shikimic Acid Dehydrogenase

Buffer:		
Tris-HCL	pH 8.0	100ml
Substrate, cofactor, stain:		
Shikimic acid	100mg	
NADP		10mg
MTT		20mg
PMS		2mg

Post staining procedure:

When the enzymatic bands were sufficiently developed the staining reaction was stopped, usually by pouring off the staining solution and fixing the gels. This was done by mixing water with acetic acid in a 5:1 ratio. Most banding patterns, however, lose some clarity with fixation or storage, and for this reason they were often scored and/or photographed. Gels were photographed using a 35mm SLR camera with ASA 125 black and white print film. A light box was used to illuminate the gels from below.

Data analysis:

The gels were scored and alleles from different populations were compared in order to test their similarity. The frequency of observed genotypes from each population was recorded.

The BIOSYS-1 computer program (Swofford and Selander 1989) was used to calculate Hardy-Weinberg equilibrium values, an index of heterozygote deficiency (Fixation test D: Wright 1965), measures of genetic similarity and dissimilarity between populations (genetic identity and distance respectively: Nei 1972) and coefficients of population differentiation (F-statistics: Wright 1965, 1978; Nei 1977; Weir and Cockerham 1984).

Deviations from the Hardy-Weinberg equilibrium were tested using a Chi-square test using Levene's (1949) correction for small sample sizes.

Wright's fixation test (F) (Wright 1965) was calculated as the ratio of the number of observed to expected heterozygotes: D = (Hobs/Hexp)-1. This is a measure of the degree of inbreeding.

The coefficients of population differentiation which were used in this study are the F-statistics which are measures of the decrease in proportion of heterozygous genotypes.

The following equations are the general forms of the three F-statistics:

$$1)\quad F_{IS} = \frac{H_S - H_I}{H_S}$$

$$2)\quad F_{ST} = \frac{H_T - H_S}{H_T}$$

$$3)\quad F_{IT} = \frac{H_T - H_I}{H_T}$$

Where:

H_I is the heterozygosity of *individuals* in a population

H_S is the expected heterozygosity of individuals in an equivalent random mating *subpopulation*

H_T is the expected heterozygosity of individuals in an equivalent random mating *total* population

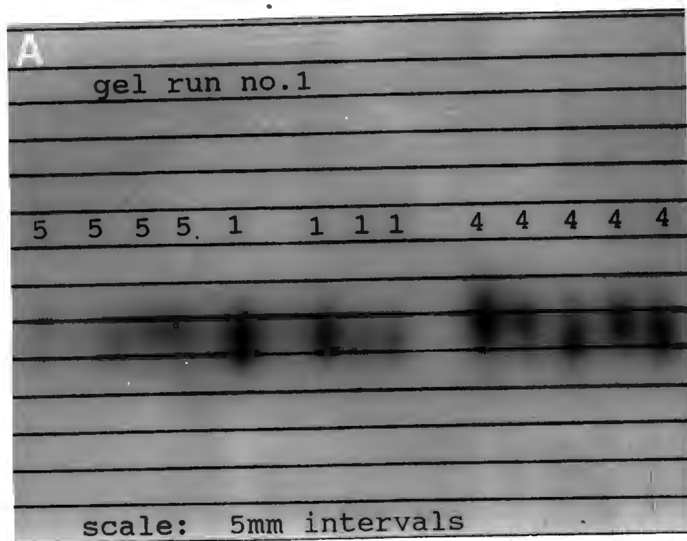
Equation 1 is thus a measure of the reduction in heterozygosity of individuals due to nonrandom mating within a subpopulation. This is referred to as the inbreeding coefficient. Equation 2 is a measure of the effects of population subdivision (fixation index), which is the reduction in heterozygosity of subpopulations due to random genetic drift. Equation 3 is the overall inbreeding coefficient of individuals which includes the effect of nonrandom mating and random genetic drift. The quantity F_{IT} measures the reduction in heterozygosity of individuals relative to the total population (Hartl 1988). BIOSYS-1 (Swofford and Selander 1989) computes these F-statistics, presenting the results in the notation of Nei (1977). The actual equations used in this analysis are Weir and Cockerhams' (1984). Unlike the other parameter estimates their F-statistics are suited to small data sets. This aids the comparisons of results of different investigators. A simple weighting procedure is used for combining the information over alleles.

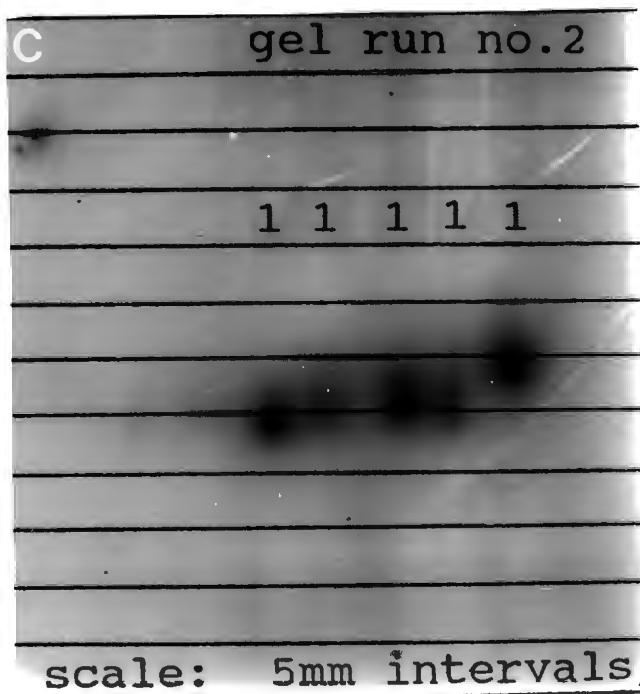
RESULTS

Different forms of alleles are produced through the process of mutation. These alleles code for the sequence in which particular amino acids are to be placed. This in turn results in the production of different forms of the same proteins or what are called isozymes. These isozymes have different electrophoretic mobilities and these are what are observed on the gels. If the individual possesses equivalent types of alleles (i.e. those inherited from the parents) then this homozygote, as it is called, is seen to produce a single darkly stained band on the gel. In the event that the allele from the mother is different from that of the fathers then two bands are observed on the gel, representing a heterozygote. This is because the proteins produced are of a different makeup and possess different electrophoretic mobilities.

This study was the first electrophoretic analysis on a species of the Cape Flora and the first on a member of the Restionaceae. It is also the first at the University of Cape Town. A number of problems were stumbled upon during the course of the study. These were mainly difficulties of interpretation of the gel banding patterns. The resolution of some banding patterns was very poor. It is clear that closer attention should be paid to the electrophoretic procedures and at least one change could be considered. That is, the use of a polyacrimide gel instead of a starch gel could greatly improve the resolution of the banding and thus increase the confidence with which the gels are scored. This paper considers the allelic diversity of a single locus only although other loci were initially analysed. They were; Malate Dehydrogenase (MDH) which showed no polymorphism and Esterase (EST) which was polymorphic. The Esterase was not analysed further owing to the very poor resolution of the bands.

The SDH isozyme is a monomer which means that it is made up of a single part. Plates A to K are photographs of some of the gels that were run. These photographs outline the types of difficulties which I came upon when interpreting the gels. The plates also indicate the letters of the alphabet used to name the alleles of different electrophoretic mobilities. It was extremely





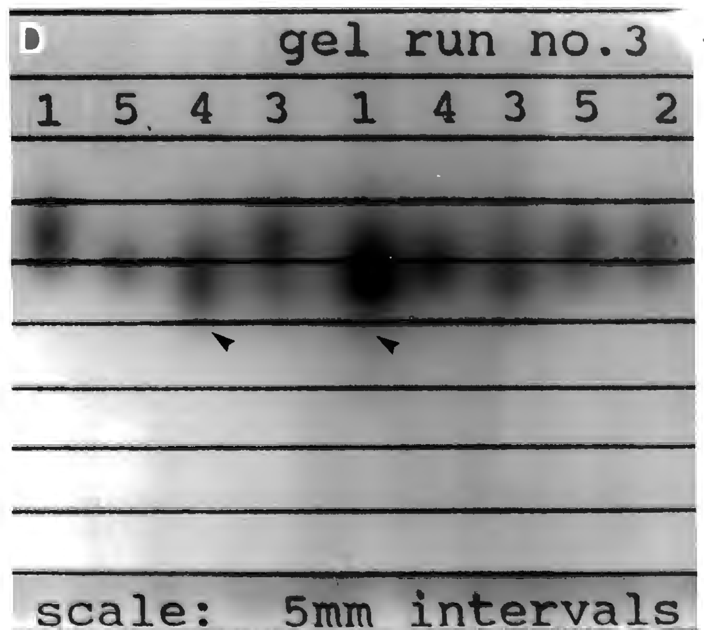
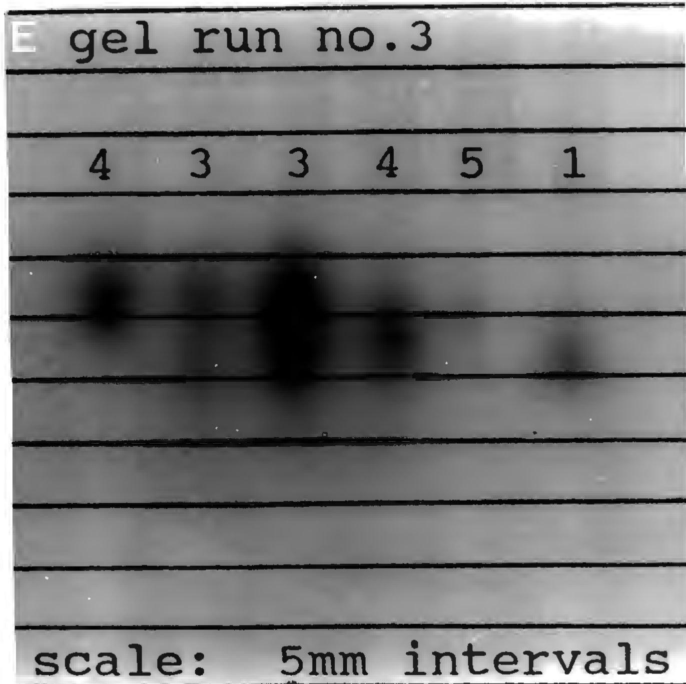
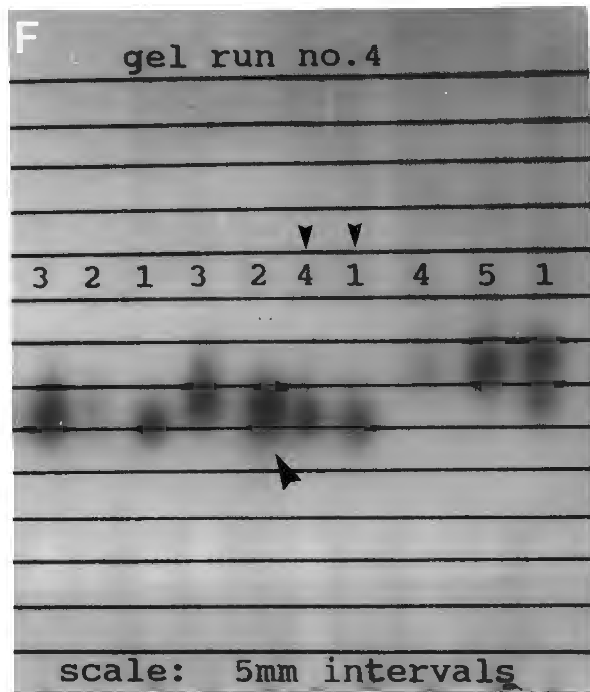
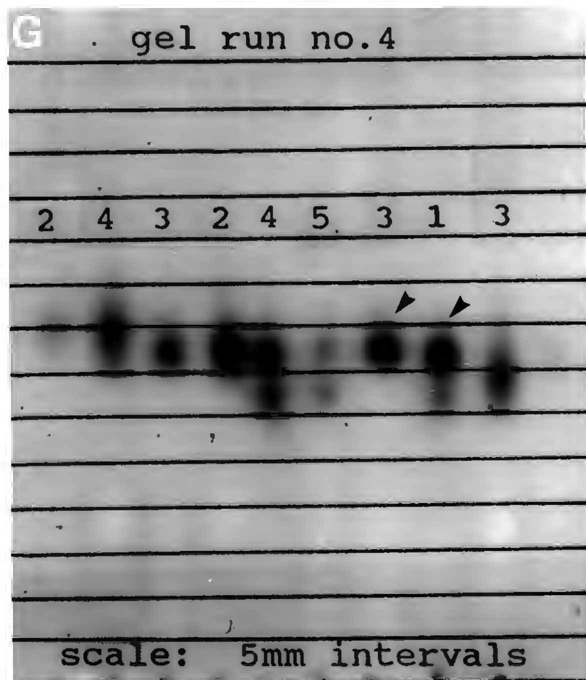


PLATE A: Population 4 consists of two heterozygotes (GH) for the fast and medium speed alleles, two heterozygotes (HI) of the slow and medium speed alleles, and possibly a homozygote (GG) for the fast allele.
PLATE B: The very slow allele from population 3 is displayed in the heterozygote form (IJ). The heterozygote (GH) of the fast and medium allele from population 3 is shown here to be equivalent to the heterozygote (GH) from population 4
PLATE C: Homozygotes of the fast (AA), medium (BB) and slow (CC) alleles for population 1
PLATE D: Possible homology of the slow allele of the possible heterozygote (BC) from population 1 with that slow allele of the heterozygote (HI) from population 4
Populations: Springfield (1), De Hoop (2), Klipfontein (3), Hectorskraal (4) and Witbakenkop (5).







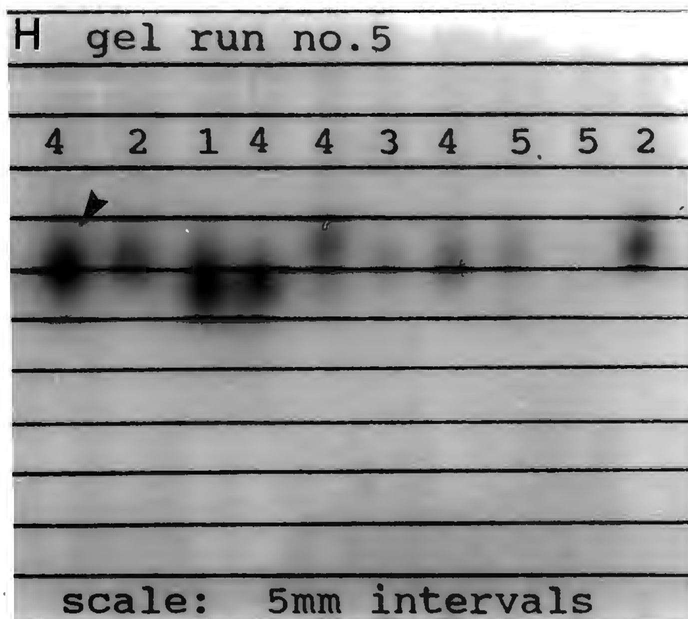
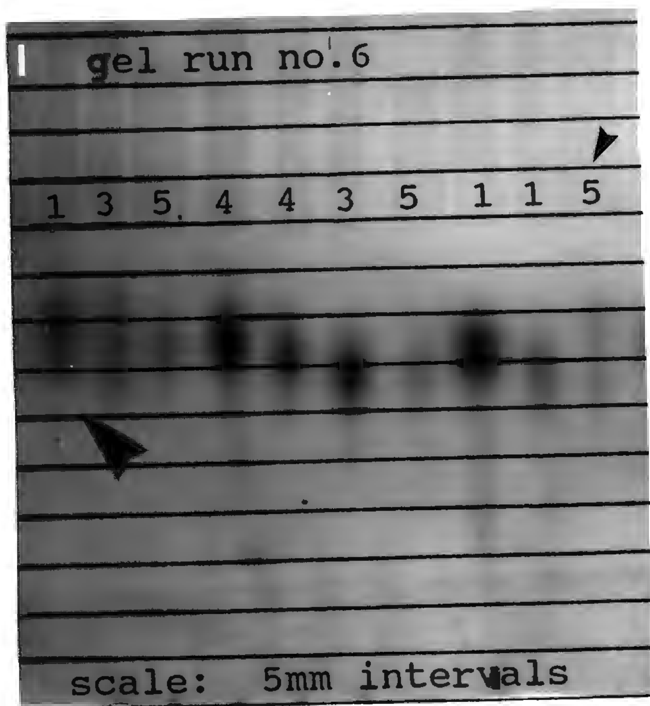
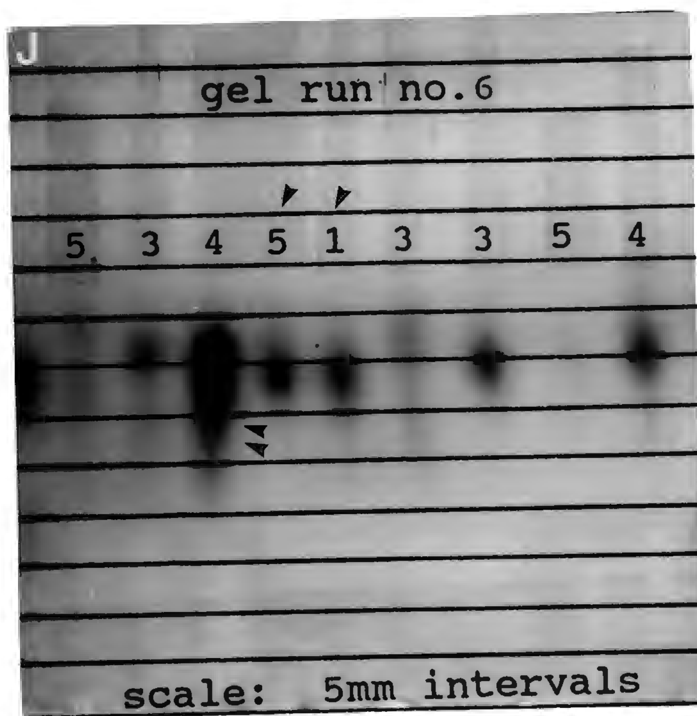


PLATE E: Two heterozygotes (GI) from population 3.
PLATE F: Homozygotes (CC) from population 1 appear not to be homologous to the homozygote (II) from population 4. The relationship of the heterozygote (EF) from population 2 to other combinations of alleles from different populations is unclear.
PLATE G: The fast alleles of the homozygote (AA) from population 1 do not appear to be homologous to those of the homozygote (GG) from population 3.
PLATE H: Uncertain identity of banding from population 4; homozygote or heterozygote ?
Populations: Springfield (1), De Hoop (2), Klipfontein (3), Hectorskraal (4) and Witbakenkop (5).





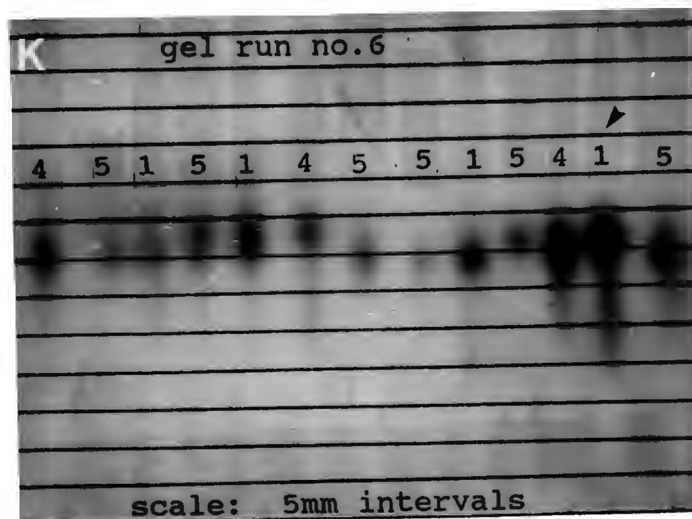


PLATE I: Faint banding of heterozygote (GI) from population 5. As with some of the other plates some bands appear to have moved much greater distances. This occurs towards the edges of the gels where presumably a different current or voltage causes this change in mobility

PLATE J: Ghost banding greatly reduces the confidence with which scoring of the gels can be undertaken. The medium speed homozygote (BB) from population 1 appears in this case to be homologous to that (HH) of population 5

PLATE K: Difficult to assertain the identity of the banding for the individual from population 1; homozygote or heterozygote ?

Populations: Springfield (1), De Hoop (2), Klipfontein (3), Hectorskraal (4) and Witbakenkop (5).

difficult to designate a form of allelic homology between the populations and so the results are presented as two parts. The first considers no allelic homology between populations at Springfield (1), De Hoop (2) and the last three populations, while the second follows a conservative approach to the interpretation of the banding patterns. By this I mean that alleles between populations considered to be different in the first analysis were considered to be homologous in the second analysis. Thus, the second part is an analysis of a "less variable" SDH locus.

SDH: high allelic diversity

Ten different alleles were recorded for the SDH locus with only Klipfontein (3), Hectorskraal (4) and Witbakenkop (5) sharing three of their alleles. Table 4 gives the allele frequencies for these five populations. The sample numbers are somewhat lower than the initial amount of individuals collected at each site. This was due to poor resolution and faint staining on the gels themselves, resulting in indecision regarding allelic identity. Tables 5 to 9 show the results of the Chi-square analyses for the deviations from Hardy-Weinberg expectations. Populations of Springfield (1) and Hectorskraal (4) are the only ones showing significant similarities to the Hardy-Weinberg equilibrium.

Coefficients of heterozygote deficiency are given in table 10 using the weighted average of F_{IS} (inbreeding coefficient) for alleles at the SDH locus. D is a similar measure, but indicates with a minus sign the deficiency of heterozygotes as a ratio of those observed to those expected. The patterns produced by both these measures are similar, indicating again that populations at Springfield (1) and Hectorskraal (4) have lower deficiencies of heterozygotes and Witbakenkop (5) having the least number of heterozygotes.
Nei's (1972) coefficients of genetic identity and genetic distance are given in tables 11 and 12, respectively. As no alleles are shared between populations at Springfield (1), De Hoop (2) and the remaining three, the values for their relationships are zero. However the populations which do share alleles show relationships which correlate with the actual distance between them.

The genetic structure of the species is summarised in tables 13 and 14. The proportion of total allelic diversity found among the populations is relatively high, as is the diversity observed within populations.

Table 4: Allele frequencies for the Springfield (1), De Hoop (2), Klipfontein (3), Hectorskraal (4) and Witbakenkop (5)populations

		Population			
	1	2	3	4	5
Alleles (N)	28	22	30	35	23
A	.071	.000	.000	.000	.000
B	.446	.000	.000	.000	.000
C	.482	.000	.000	.000	.000
D	.000	.455	.000	.000	.000
E	.000	.227	.000	.000	.000
F	.000	.318	.000	.000	.000
G	.000	.000	.267	.286	.304
H	.000	.000	.383	.343	.348
I	.000	.000	.283	.371	.348
J	.000	.000	.067	.000	.000

Table 5: Observed and expected genotype frequencies for Springfield, with a Chi-square test for deviation from Hardy-Weinberg equilibrium using Levene's (1949) correction for small sample size

Class	Observed frequency	Expected frequency	Chi-square	DF	P
A-A	0	0.1			
A-B	3	1.8			
A-C	1	2.0			
B-B	6	5.5			
B-C	10	12.3			
C-C	8	6.4			
			2.236	3	.525

Table 6: Observed and expected genotype frequencies for De Hoop, with a Chi-square test for deviations from the Hardy-Weinberg equilibrium using Levene's (1949) correction for small sample size

Class	Observed frequency	Expected frequency	Chi-square	DF	P
D-D	7	4.4			
D-E	4	4.7			
D-F	2	6.5			
E-E	2	1.0			
E-F	2	3.3			
F-F	5	2.1			
			10.008	3	.019

Table 7: Observed and expected genotype frequencies for Klipfontein, with a Chi-square test for deviations from Hardy-Weinberg equilibrium using Levene's correction for small sample size

Class	Observed frequency	Expected frequency	Chi-square	DF	P
G-G	5	2.0			
G-H	3	6.2			
G-I	3	4.6			
G-J	0	1.1			
H-H	7	4.3			
H-I	4	6.6			
H-J	2	1.6			
I-I	4	2.3			
I-J	2	1.2			
J-J	0	0.1			
			12.505	6	.052

Table 8: Observed and expected genotype frequencies for Hectorskraal, with a Chi-square test for deviations from Hardy-Weinberg equilibrium using Leven's (1949) correction for small sample size

Class	Observed frequency	Expected frequency	Chi-square	DF	P
G-G	4	2.8			
G-H	6	7.0			
G-I	6	7.5			
H-H	5	4.0			
H-I	8	9.0			
I-I	6	4.7			
			1.732	3	.630

Table 9: Observed and expected genotype frequencies for Witbakenkop, with a Chi-square test for deviations from Hardy-Weinberg equilibrium using Levene's (1949) correction for small sample size

Class	Observed frequency	Expected frequency	Chi-square	DF	P
G-G	4	2.0			
G-H	3	5.0			
G-I	3	5.0			
H-H	6	2.7			
H-I	1	5.7			
I-I	6	2.7			
			15.704	3	.001

Table 10: Coefficients for heterozygote deficiency or excess for all five populations

Population	Obs. het.	Exp. het.	Fixation index $F_{IS(i)}$	D
1 Springfield	14	16.1	.112	-.128
2 De Hoop	8	14.4	.432	-.445
3 Klipfontein	14	21.3	.331	-.342
4 Hectorskraal	20	23.6	.138	-.150
5 Witbakenkop	7	15.6	.543	-.553

D = (Het. Obs./Het. Exp.-1) (Wright's (1965) fixation test)
Fixation index $F_{IS(i)}$ is the weighted average of F_{IS} for all alleles at the SDH locus in the $_{i}$(th) subpopulation

Table 11: Matrix of single-locus (SDH) genetic identity coeficients (Nei 1972)

	Population	1	2	3	4	5
1	Springfield	*****				
2	De Hoop	.000	*****			
3	Klipfontein	.000	.000	*****		
4	Hectorskraal	.000	.000	.979	*****	
5	Witbakenkop	.000	.000	.984	.999	*****

Table 12: Matrix of single-locus (SDH) genetic distance coeficients (Nei 1972)

	Population	1	2	3	4	5
1	Springfield	*****				
2	De Hoop	.000	*****			
3	Klipfontein	.000	.000	*****		
4	Hectorskraal	.000	.000	.021	*****	
5	Witbakenkop	.000	.000	.017	.003	*****

Table 13: The $F_{IS(ik)}$ statistic for each allele of the SDH locus, with the weighted average of F_{IS} for all alleles at the SDH locus in the $_i$(th) population

	Subpopulation				
Allele	1	2	3	4	5
A	-.077				
B	.061				
C	.213				
D		.450			
E		.224			
F		.581			
G			.489	.160	.384
H			.365	.112	.617
I			.261	.143	.617
J			-.071		
$F_{IS(i)}$	.112	.432	.331	.138	.543

Table 14: The $F_{IS(k)}$, $F_{IT(k)}$ and $F_{ST(k)}$-statistics for each allele of the SDH locus. ($F_{IS(k)}$ values are the weighted averages of $F_{IS(ik)}$ across subpopulations)

Allele	$F_{IS(k)}$	$F_{IT(k)}$	$F_{ST(k)}$
A	-.077	-.014	.058
B	.061	.429	.392
C	.213	.549	.427
D	.450	.670	.400
E	.224	.371	.190
F	.581	.695	.272
G	.343	.434	.139
H	.365	.482	.184
I	.341	.455	.172
J	-.071	-.014	.054

Weighted averages of F-statistics above for SDH locus

F_{IS}	F_{IT}	F_{ST}
.317	.482	.242

SDH: low allelic diversity

The following results assumed the alleles of populations at Springfield (1) and De Hoop (2) to be homologous to each other and homologous to those of the remaining populations. Accordingly, the total allelic diversity is somewhat lower, with only 4 allelomorphs present for the analysis. The allelic frequencies are given in table 15. As far as the Chi-square analyses of the deviations from Hardy-Weinberg equilibrium within populations are concerned, the results are the same as the first analysis.

Table 15: Allele frequencies for the SDH locus (a conservative interpretation of the gels)

	Population				
	SPRI	DE HO	KLIP	HECT	WITK
(N)	28	22	30	35	23
A	.071	.455	.267	.286	.304
B	.446	.227	.383	.343	.348
C	.482	.318	.283	.371	.348
D	.000	.000	.067	.000	.000

Matrices of Nei's (1972) coefficients of genetic identity and genetic distance are given in table 16 and 17 respectively. The patterns seem quite complex with some observable relation to distance between sites.

The genetic structure of the species is summarised by the F-statistics in tables 18 and 19. The proportion of total allelic diversity found among the populations is considerably lower, as is the total allelic diversity. Genetic variation within populations remained unchanged from the first analysis.

DISCUSSION

Deviations from Hardy-Weinberg Equilibrium

Genotype trigonomics, as measured by isozyme analysis, can give an indication of the breeding biology of a species. Heterozygote deficiencies typically indicate a measure of selfing. In this study heterozygote deficiencies wre found in the populations at De Hoop (2), Klipfontein (3) and Witbakenkop (5). This implies inbreeding in these populations. Since the species is dioecious, this is unlikely to be the case. Possble alternative explanations for the departures of genotype trigonomics from Hardy-Weinberg equilibrium are that either the result is not representative of the population as a whole or problems exist when interpreting the banding on the gel.

Homozygote selective advantage may be used to explain these heterozygote deficiencies. That is any combination of alleles which has some advantage will be selected for. Perhaps, through pleiotropy the variation in the isozymes of SDH is in some way connected to the phenology of the individual populations. This is requires confirmation. However, this kind of argument is disreputed by proponents of the theory of neutral selection for isozymes (Hartl 1988).

Population differentiation

The fixation index F_{ST} or the proportion of total allelic diversity among populations (table 15) is much higher than that predicted for dieocious wind pollinated species (Hamrick and Godt 1990). This could be expected if only some of the populations share alleles, namely Klipfontein (3), Hectorskraal (4) and Witbakenkop (5). If this value is realistic, then there is a barrier to gene flow between Springfield (1) and De Hoop (2) and the three eastern populations.

It has been argued that only occasional gene flow is needed to prevent the differentiation of neutral alleles (Loveless and Hamrick 1984). In this case the barriers to gene flow must have existed for many generations, causing the loss of

ancestral alleles through random genetic drift. Barriers to gene flow may be phenological, indicating shifts in flowering time or they may be geographic in nature. Simple isolation by distance may have caused the lack of gene flow between populations. The Breede river valley itself may act as a barrier to gene flow between the populations east and west of the valley. As far as flowering times are concerned no data is available.

If one takes a conservative view of the allelic diversity the picture is quite different. The proportion of allelic diversity among populations is dramatically lower (table 19). For this second analysis, F_{ST} value was 0.038 overall, indicating low levels of genetic drift. Perhaps the reality lies somewhere between this conservative value and that of the initial analysis. G_{ST} values of 0.099 on average are found for outcrossing wind pollinated taxa (Hamrick and Godt 1990), indicating low levels of genetic drift among populations. However, as discussed in the opening paragraphs, taxa having narrow geographical ranges, such as *T. insignis*, may be expected to have relatively high G_{ST} values (i.e. F_{ST} equivalent) (Hamrick and Godt 1990).

The proportion of allelic diversity found within populations (F_{IS}) (table 19) is high overall (i.e. 0.317). This implies that the genetic neighbourhood sizes are relatively small, with reduced gene flow within populations. This is not the prediction emerging from the work of Honig *et al.* (1992) on *Staberoha banksii* for which large genetic neighbourhoods are found. They found that wind pollination was highly effective as a vector, with high pollen loads on the pistils of female plants, and hence, considerable potential for gametophytic competition (Honig *et al.* 1992). Whether this type of competition has some effect on the maintainence of allelic diversity within populations of *Thamnochortus insignis*, it is not known. As far as seed set is concerned, *Staberoha banksii* plants situated up to "13m away from the nearest male plant showed no significant decline in seed set, which is approximately four to six times the average distance separating the sexes" (Honig *et al.* 1992). They suggest that there may be considerable mixing of airborne pollen from different males, and that gene flow within populations is high.

Gene flow in the *T. insignis* populations may indeed be high and it is the large population sizes which prevent random genetic drift from lowering the allelic diversity. Alternatively, SDH may be the only locus showing such high allelic diversity. Most isozyme studies do in fact consider many loci, and it is the average allelic diversity which is most often presented in published works (eg. Hamrick and Godt 1990).

Nei's genetic identity and distance coefficients of the first data analysis show that Hectorskraal (4) and Witbakenkop (5) have the most similar genetic structure, with Klipfontein (3) being more similar to Witbakenkop (5) than Hectorskraal (4) (tables 11 and 12). The values themselves are very high suggesting that there is very little difference in genetic structure. That Hectorskraal (4) is most similar to Witbakenkop (5) is not surprising, on the basis of their close proximity to each other (figure 1). The matrices produced by the second analysis again show that Hectorskraal (4), Klipfontein (3) and Witbakenkop (5) are very similar genetically (tables 16 and 17). However, it is surprising that Springfield (1) is more similar to Hectorskraal (4), Klipfontein (3) and Witbakenkop (5), than it is to De Hoop (2) (figure 2) since De Hoop (2) is spatially closer to the eastern populations. The predictive strength of these similarity values is greatly reduced if one considers that only a single locus is under study. Further study of more loci is needed to confirm this pattern of genetic relatedness.

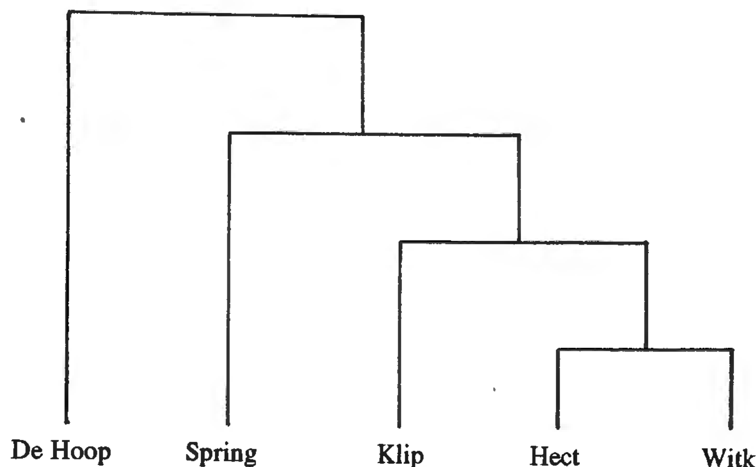


Figure 2: A phenogram of Nei's (1972) coeficients of genetic identity for the five populations. Springfield (Spring), Klipfontein (Klip), Hectorskraal (Hect) and Witbakenkop (Witk)

CONCLUSION

I have discussed the limitations of a population genetic analysis based on a single locus. These limitations pertain to the genetic simmilarity and dissimilarity coeficients of Nei (1972) and not to the coeficients of inbreeding and genetic drift. It seems that this limitation to a single locus is not seen as being that important. For example, Brain (unpubl.) used a single locus in a genetic analysis of *Acacia karroo*. However, much can and has been said regarding the distribution of the allelic variation of SDH throughout the range of this species. Differences in the results which occurred when both a conservative estimate and critical method of gel interpretation were undertaken, have also been discussed. In these cases, it is suggested, the reality may lie somewhere inbetween. This is possibly true for the proportion of total allelic variation found among the populations, than would be found if more loci were considered.

For the initial analysis the populations are genetically differentiated dispite wind pollination. If the flowering time for each population was different then it would present evidence of a barrier to gene flow. Isolation by distance may also play a role in creating a barrier to gene flow. This is shown by the neighbouring populations in the east, between which differentiation is minimal, as would be predicted by their close proximity.

In the light of the work carried out here and considering the deviations from theory predicted for wind pollinated obligate outcrossers, it would be interesting to further the current research path, in search of other polymorphic loci. This, paired with a closer monitoring of the electrophoretic procedure may produce clearer and more easily interpretable results.

Acknowledgements
Janet Thomas is thanked for her encouragement and technical and theoretical assistance. William Bond is thanked for useful comments on the manuscript. Peter Linder is thanked for his patience "Down Under". Rene Schoenegevel is thanked for her help with the lab work. Last but not least, Jeronamo the dog.

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