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# Allozyme Variation in *Virgilia oroboides* (Tribe Podalyrieae: Fabaceae)

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**Key Word Index**—Fabaceae; Cape legumes; *Virgilia oroboides*; genetic diversity; protein electrophoresis.

**Abstract**—Horizontal starch gel electrophoresis was used to examine genetic diversity within a morphologically uniform population of *Virgilia oroboides*. Fifty trees were surveyed for 37 proteins, and gene products of 43 protein coding loci revealed genetic variation at 21 (48.8%) thereof. The percentage of polymorphic loci was 46.51% (0.95 criterion), a value of 1.49 (±0.08) was obtained for the mean number of alleles per locus, and the average heterozygosity per locus was calculated at 0.207 (±0.034). Although the mean heterozygosity per locus is relatively high for plant species in general, it compares favourably with values obtained for vascular plants, plants with comparable life history characteristics and other legumes.

## Introduction

*Virgilia* is a small genus of papilionoid legumes endemic to the southwestern and southern coastal areas of South Africa, from where it has been introduced as ornamental trees to many parts of the world. As forest margin relicts, the species are of considerable taxonomic and ecological interest.

In the latest available taxonomic treatment (Van Wyk, 1986), three allopatric taxa were recognised, namely *V. oroboides* (Berg.) Salter ssp. *oroboides*, *V. oroboides* ssp. *ferruginea* B.-E. van Wyk and *V. divaricata* Adamson. Each taxon comprises a number of geographically isolated populations, which show limited morphological variation within populations but distinct differences between populations. This pattern of variation was demonstrated by discriminant analysis (Van Wyk, 1983). There was also subtle discontinuities in secondary metabolites, notably alkaloids (Greinwald *et al.*, 1989; Veen *et al.*, 1991) and flower anthocyanins (Van Wyk and Winter, 1994), but these chemical characters are generally too conservative to be of much value at the population level. The overall pattern of variation in *Virgilia* is typical of most Cape legumes and suggests allopatric speciation. *Virgilia* is pollinated by xylocopid bees, which are known to operate over distances of a few kilometres at the most. Pollen transfer between populations may further be limited by geographical isolation and by differences in the time of flowering (spring vs summer).

Starch gel electrophoresis provided plant biologists with additional markers with which to study evolutionary processes and isozyme loci have several advantages over single-gene morphological traits. These advantages include: (1) the demonstration of genetic (Mendelian) inheritance of electrophoretically detected traits; (2) the direct calculation of allele frequencies; (3) the direct comparison of estimates of levels and distribution of genetic variation between populations and species; (4) the assaying of an array of enzymatic loci using small quantities of material such as leaves; and (5) probably most important, isozymes can be resolved for most plant species regardless of life cycle, habitat, size or longevity (Hamrick, 1989). The electrophoretic data can

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also be utilised to study phylogenetic relationships among taxa and it offers the opportunity for genetic improvements and selective breeding of legumes that was formerly unavailable. The feasibility of using enzyme electrophoresis to describe and quantify genetic diversity within Cape legumes, and *Virgilia* in particular, is reported in this paper.

## Materials and Methods

*Plant material.* Leaf and flower samples were compared and the former produced higher concentrations of enzyme activity. Leaf samples from 50 trees of *V. oroboides* ssp. *oroboides* were then collected from a natural population in Tradouws Pass (latitude: 33°57'S; longitude: 20°42'E; altitude: 340 m), between Swellendam and Barrydale in the southern Cape province of South Africa. This population was chosen because it is geographically isolated from other taxa of *Virgilia* (both natural and cultivated) so that there is no possibility of hybridisation or introgression. Furthermore, this population is morphologically uniform, suggesting low levels of outcrossing with other populations.

*Procedure.* Young leaves from actively growing shoots were collected in cryotubes and immediately submerged in liquid nitrogen (−196°C). Two grams of leaf tissue was extracted in 2 ml of Tris–HCl buffer (pH = 7.5), described by Soltis *et al.* (1983), by manually grinding it with a glass mortar and pestle on ice. The liquid was transferred to Eppendorf test tubes, centrifuged at 4000 *g* for 5 min and the supernatant absorbed onto paper wicks for starch gel electrophoresis. Recommendations by Kephart (1990) were taken into consideration to optimise electrophoretic conditions. Six gels could be run simultaneously (using a sample twice only and staining for up to 23 enzyme and protein systems) to minimise the effect of thawing.

Twelve per cent starch (Sigma: S-4501) gels were used and 25 of the 37 enzyme and protein systems produced interpretable banding patterns (Table 1). Genetic interpretation of enzyme banding patterns was based on the subunit structure of the enzymes (Gottlieb, 1981, 1982; Kephart, 1990). Locus nomenclature followed Harris and Hopkinson (1976), Soltis and Soltis (1989), Hillis and Moritz (1990) and Shaklee *et al.* (1990). Locus abbreviations, monomorphic loci and enzyme commission numbers are given in Table 1, and buffer system combinations giving the best results are listed in Table 2. Statistical analysis of allozyme data was executed using *BIOSYS-1* (Swofford and Selander, 1981).

TABLE 1. LOCUS ABBREVIATIONS AND ENZYME COMMISSION NUMBERS (E.C. No.) ARE LISTED AFTER EACH PROTEIN (* = monomorphic loci)

| Protein | Locus | E.C. No. |
|---|---|---|
| Acid phosphatase | (*ACP-1, -2*) | 3.1.3.2 |
| Adenylate kinase | *(*AK*) | 2.7.4.3 |
| Aspartate aminotransferase | (*AAT*) | 2.6.1.1 |
| Cytosol aminopeptidase | *(*CAP-1, -3*) | 3.4.11.1 |
| | (*CAP-2*) | |
| Dihydrolipoamide dehydrogenase | (*DDH*) | 1.8.1.4 |
| Esterase | *(*EST-1, -2, -5, -6, -8*) | 3.1.1.– |
| | (*EST-3, -4, -7, -9*) | |
| General protein | *(*PROT-1, -2*) | |
| Glyceraldehyde-3-phosphate dehydrogenase | (*GAPDH*) | 1.2.1.12 |
| Glucose-6-phosphate isomerase | (*GPI*) | 3.5.1.9 |
| Guanine deaminase | *(*GDA*) | 3.5.4.3 |
| Isocitrate dehydrogenase | *(*IDH*) | 1.1.1.42 |
| Leucine aminopeptidase | (*LAP*) | 3.4.11.1 |
| Malate dehydrogenase | *(*MDH-1, -2*) | 1.1.1.37 |
| Malic enzyme | *(*ME*) | 1.1.1.38 |
| Menadione reductase | *(*MNR-1, -2*) | 1.6.99.– |
| Peptidase: | | 3.4.–.– |
| Substrate: Glycyl-L-leucine | *(*PEP-A*) | |
| Leucylglycylglycine | (*PEP-B*) | |
| Leucine-alanyl | (*PEP-C*) | |
| L-Phenylalanyl-L-proline | *(*Pep-D,1, -D,2*) | |
| Leucyl-tyrosine | (*PEP-S*) | |
| Peroxidase | (*PER-1, -2*) | 1.11.1.7 |
| 6-Phosphogluconate dehydrogenase | (*PGD*) | 1.1.1.44 |
| Purine-nucleoside phosphorylase | *(*NP-1, -2*) | 2.4.2.1 |
| Shikimate dehydrogenase | (*SKDH*) | 1.1.1.25 |
| Superoxide dismutase | (*SOD-1, -2*) | 1.15.1.1 |

TABLE 2. PROTEIN AND BUFFER SYSTEM COMBINATIONS

| | Molarity | | Activity | |
|---|---|---|---|---|
| Reference | Electrode | Gel | High | Moderate |
| Cooke and Buckley (1987) | pH = 8.1<br>LiOH 0.03<br>Boric acid 0.19 | pH = 8.4<br>Citric acid 0.008<br>Tris 0.05 | *CAP*<br>*DDH*<br>*NP* | |
| Markert and Faulhaber (1965) | pH = 8.6<br>Tris 0.18<br>EDTA 0.004<br>Boric acid 0.1 | 1:4 | *AAT*<br>*GAPDH*<br>*LAP*<br>*MNR*<br>*PEP-B, -C, -S*<br>*PER*<br>*PROT*<br>*SKDH* | *DDH*<br>*ME*<br>*MDH*<br>*PEP-D* |
| Clayton and Tretiak (1972) | pH = 6.1<br>Citric acid 0.04<br>Morpholine adjust | 1:19 | *ACP*<br>*CAP*<br>*GDA* | *GAPDH* |
| Poulik (1957) | pH = 8.2<br>NaOH 0.05<br>Boric acid 0.03 | pH = 8.7<br>Tris 0.076<br>Citric acid 0.005 | *GPI*<br>*PER*<br>*SOD* | *AAT*<br>*GPI*<br>*LAP*<br>*PEP-B, -C, -D, -S* |
| Ridgway *et al.* (1970) | pH = 8.0<br>LiOH 0.06<br>Boric acid 0.3 | pH = 8.7<br>Tris 0.3<br>Citric acid 0.05<br>10% electrode | *EST*<br>*GPI*<br>*ME*<br>*PEP-A, -D*<br>*PGD*<br>*SOD* | *AK*<br>*AAT*<br>*LAP*<br>*MNR*<br>*PEP-C, -D, -S*<br>*PGM* |
| Whitt (1970) | pH = 6.9<br>Tris 0.3<br>Citric acid 0.1 | 1:29 | *ACP*<br>*AK*<br>*MDH* | *CK*<br>*DDH*<br>*GAPDH*<br>*GPI*<br>*GDA*<br>*IDH*<br>*LAP* |

## Results

Forty-three protein coding loci provided interpretable results in *V. oroboides*, of which 46.51 (95% criterion) displayed polymorphism. Twenty-two of the 43 loci (51.2%) displayed monomorphic gel banding patterns (Table 1) and products of the following loci migrated cathodally: *ACP-2, CAP-3, EST-7, -8, -9, GDA, PER-2* and *SOD-2*. In addition to these loci, we stained for aconitase hydratase (E.C. 4.2.1.3), alcohol dehydrogenase (E.C. 1.1.1.1), catalase (E.C. 1.11.1.6), creatine kinase (E.C. 2.7.3.2), fumarate hydratase (E.C. 4.2.1.2), glucose-3-phosphate dehydrogenase (E.C. 1.1.1.8), hexokinase (E.C. 2.7.1.1), L-iditol dehydrogenase (E.C. 1.1.1.14), L-lactate dehydrogenase (E.C. 1.1.1.27), mannose-6-phosphate isomerase (E.C. 5.3.1.8), phosphoglucomutase (E.C. 5.4.2.2) and general (unidentified) protein using amido black. These enzymes and proteins, together with *AK-2, GPI-2* and *IDH-2*, did not show sufficient activity or resolution to score it satisfactorily in *V. oroboides* samples. General protein (*PROT-1, -2*) activity was high when stained with Coomassie blue. Most of the enzymes lost their activity after thawing it the second time, resulting in less individuals which could be scored routinely.

Table 3 presents allele frequencies and Chi-square ($\chi^2$) values for polymorphic loci. Loci where significant ($P<0.05$) deviations of alleles from expected Hardy–Weinberg proportions occurred and individual heterozygosities are also listed in Table 3.

TABLE 3. SAMPLE SIZE, ALLELE FREQUENCIES, OBSERVED NUMBER OF HETEROZYGOTES (*OBS*), COEFFICIENTS FOR HETEROZYGOSITY DEFICIENCY OR EXCESS (*D*), $\chi^2$ VALUES, DEGREES OF FREEDOM (d.f.), AND INDIVIDUAL HETEROZYGOSITIES (*h*) FOR POLYMORPHIC LOCI

| Locus | *N* | fA | fB | *OBS* | *D* | $\chi^2$ | d.f. | *h* |
|---|---|---|---|---|---|---|---|---|
| *ACP-1* | 36 | 0.375 | 0.625 | 9 | −0.467 | 7.840* | 1 | 0.469 |
| *ACP-2* | 50 | 0.180 | 0.820 | 4 | −0.729 | 26.572* | 1 | 0.295 |
| *AAT* | 49 | 0.643 | 0.357 | 17 | −0.244 | 2.928 | 1 | 0.459 |
| *CAP-2* | 50 | 0.600 | 0.400 | 16 | −0.333 | 5.556* | 1 | 0.480 |
| *DDH* | 35 | 0.471 | 0.529 | 25 | 0.433 | 6.570* | 1 | 0.498 |
| *EST-3* | 34 | 0.559 | 0.441 | 14 | −0.165 | 0.925 | 1 | 0.493 |
| *EST-4* | 12 | 0.292 | 0.708 | 3 | 0.395 | 1.872 | 1 | 0.413 |
| *EST-7* | 42 | 0.952 | 0.048 | 2 | −0.475 | 9.476* | 1 | 0.091 |
| *EST-9* | 35 | 0.629 | 0.371 | 8 | −0.510 | 9.121* | 1 | 0.467 |
| *GAPDH* | 10 | 0.250 | 0.750 | 1 | −0.733 | 5.378* | 1 | 0.375 |
| *GPI* | 50 | 0.770 | 0.230 | 11 | 0.379 | 7.178* | 1 | 0.354 |
| *LAP* | 50 | 0.550 | 0.450 | 13 | −0.475 | 11.269* | 1 | 0.495 |
| *PEP-B* | 50 | 0.490 | 0.510 | 17 | −0.320 | 5.111* | 1 | 0.500 |
| *PEP-C* | 41 | 0.341 | 0.659 | 10 | −0.458 | 8.588* | 1 | 0.450 |
| *PEP-S* | 47 | 0.436 | 0.564 | 25 | 0.081 | 0.312 | 1 | 0.492 |
| *PER-1* | 49 | 0.551 | 0.449 | 22 | −0.093 | 0.420 | 1 | 0.495 |
| *PER-2* | 47 | 0.340 | 0.660 | 14 | 0.337 | 5.328* | 1 | 0.449 |
| *PGD* | 19 | 0.474 | 0.526 | 6 | −0.367 | 2.554 | 1 | 0.499 |
| *SKDH* | 41 | 0.402 | 0.598 | 23 | 0.166 | 1.135 | 1 | 0.481 |
| *SOD-1* | 30 | 0.833 | 0.167 | 4 | −0.520 | 8.112* | 1 | 0.278 |
| *SOD-2* | 37 | 0.243 | 0.757 | 4 | −0.706 | 18.460* | 1 | 0.368 |

* = Loci where significant ($P<0.05$) deviations of alleles from expected Hardy–Weinberg proportions occurred.

Allozyme phenotypes of putative heterozygotes at loci were congruent with those expected on the basis of the quaternary structure of the enzyme (Ward, 1977). Thus heterozygotes at *AAT, GPI, PGD* and *SOD* were triple banded, as expected for dimeric enzymes, five banded at the tetrameric locus (*GAPDH*), and double banded at the monomeric enzymes: *ACP, CAP, DDH, EST, LAP, PEP, PER* and *SKDH* (Table 3).

Genotypic frequencies at seven loci (*AAT, EST-3, -4, PEP-S, PER-1, PGD* and *SKDH*) in *V. oroboides* (Table 3) closely approximated Hardy–Weinberg expectations. Relatively large deficiencies of heterozygotes and deviations of allele frequencies from expected Hardy–Weinberg proportions were encountered at the *ACP-1, -2, EST-7, -9, GAPDH, LAP, PEP-C, SOD-1* and *-2* protein coding loci (Table 3). The mean number of alleles per locus (*A*) was 1.49 (±0.08), individual heterozygosity values (*h*) ranged from 0.091 to 0.500, and the average heterozygosity (*H*) value was 0.207 (±0.034).

## Discussion

Deviations from expected Hardy–Weinberg proportions occurred at the *ACP, DDH, EST-7, -9, GAPDH, GPI, LAP, PER-1, PEP-C* and *SOD* loci (Table 3). A deficit of heterozygotes did not occur at the *DDH, PEP-S* and *SKDH* protein coding loci. Hardy–Weinberg proportions of allele frequencies were obtained at the *AAT, EST-3, -4, PEP-1, PEP-S, PGD* and *SKDH* loci. Various factors can shift the equilibrium and disrupt the stability of a population, giving rise to change in the genetic structure. Van der Bank (in press) gives an overview of such factors. The extent of the effect that such factors could have had on *V. oroboides* is unknown since very little is known about their biology. It is, however, interesting to note that the sample size for all but one of the loci where large differences of allele frequencies from expected Hardy–

Weinberg proportions occurred, *GAPDH*, was 30 or more (Table 3). Rare and/or scarce alleles are therefore present at these loci.

Previous studies on plants reported the percentage of polymorphic loci (*P*) of 37%, *A* of 1.69, and an *H* value per individual of 0.141 (Hamrick *et al.*, 1979). The values obtained in the present study (46.5%, 1.49, 0.207, respectively) differ from these values and the latter value is also higher than the mean value obtained for dicots in general (0.113), as estimated by Hamrick *et al.* (1979) from 74 taxa. Nevertheless, these values fall within the range of means reported (Hamrick, 1979) for vascular plants in general ($P=22.0–75.3\%$, $A=1.35–2.56$, $H=0.079–0.354$). Levels of genetic variation have also been correlated with various ecological and live-history traits in plants. For example, our estimate of *H* compares favourably to results for plants utilising animals in their breeding system ($H=0.187$) and also for explosive seed dispersal mechanisms ($H=0.262$) (Loveless and Hamrick, 1984). Hamrick (1979) and Hamrick *et al.* (1979) also obtained high levels of genetic variation within populations of long-lived species, presumably because of large numbers of generations present in any given population (Levin, 1978).

Different values obtained in various studies can be accounted for by the analysis of different species, sample sizes, increased number of loci now studied, and the choice of loci which may have differences in heterozygosities (Lewontin, 1974). For example, *h* values calculated from allele frequencies provided by Liston (1992) for eight *Astragalus* species (Fabaceae, tribe Galegeae) ranged from 0.000 to 0.199. It compares favourably with *H* values obtained in the present study (Table 3). Small *et al.* (1992) and Koenig and Gepts (1989), on the other hand, obtained limited isozyme variation ($H=0.011–0.058$ and $H=0.132$, respectively). Our estimates are, however, congruent with values obtained by: (1) Godt and Hamrick (1991), who studied 32 populations of *Lathyrus latifolius* (Fabaceae, tribe Phaseoleae) and obtained $P=54\%$ (range $=28.6–71.4\%$), $A=1.38$ and $H=0.207$, (2) Pasquet (1993) who obtained $H=0.071–0.307$; $P=16–78\%$ and $A=2.0–3.24$, and (3) Schinkel and Gepts (1989) who reported an *H* value of 0.247.

In conclusion, this is the first account of electrophoretic variants of *V. oroboides.* The results of the present study indicate that the species examined possesses a sufficient amount of genetic variation to allow them to adapt to environmental changes or for them to be used in selection programmes. No genetic selection of *Virgilia* has been done at this stage to obtain favourable phenotypes (e.g. to be used as ornamental trees). The higher levels of genetic variation obtained in the present study are also not unexpected when compared to values reported for the majority of species from the same family, and to plant species with similar life history characteristics. The isozymes described in this study, therefore, provide a good basis for estimating the amount and pattern or distribution of genetic variation within this species and the enzymatic polymorphism revealed interesting data for both fundamental and applied research. Also, direct impacts such as habitat destruction and displacements of native flora have the potential to induce profound ecological changes. These effects can, however, be reduced by appropriate nature management to shelter stocks (especially when the genetic integrity of populations or the species is involved). The identification of putative hybrids may also be of value in conservation management, where cultivated trees might have led to genetic contamination of natural populations. We propose that a wider survey of all the taxa (including samples from various geographical regions and morphologically distinct populations) may provide an even better understanding of the genetic divergence and biogeography of the genus *Virgilia.*

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## References


Clayton, J. W. and Tretiak, D. N. (1972) Amine-citrate buffers for pH control in starch gel electrophoresis. *J. Fish. Res. Board Can.* **29**, 1169–1172.

Cooke, F. and Buckley, P. A. (1987) *Avian Genetics.* Academic Press, London.

Godt, M. J. and Hamrick, J. L. (1991) Genetic variation in *Lathyrus latifolius* (Leguminosae). *Am. J. Bot.* **78**, 1163–1171.

Gottlieb, L. D. (1981) Electrophoretic evidence and plant populations. *Prog. Phytochem.* **7**, 1–46.

Gottlieb, L. D. (1982) Conservation and duplication of isozymes plants. *Science* **216**, 373–380.

Greinwald, R., Veen, G., Van Wyk, B.-E., Witte, L. and Czygan, F.-C. (1989) Distribution and taxonomic significance of major alkaloids in the genus *Virgilia*. *Biochem. Syst. Ecol.* **17**, 231–238.

Hamrick, J. L. (1979) Genetic variation and longevity. In *Topics in Plant Population Biology* (Solbrig, O. T., Jain, S., Johnson, G. B. and Raven, P. H., eds), pp. 84–113. Columbia University Press, New York.

Hamrick, J. L. (1989) Isozymes and the analysis of genetic structure in plant populations. In: *Isozymes in Plant Biology* (Soltis, D. E. and Soltis, P. S., eds), Chapter 4, pp. 87–105. Chapman and Hall, London.

Hamrick, J. L., Linhart, Y. B. and Mitton, J. B. (1979) Relationships between life history characteristics and electrophoretically-detectable genetic variation in plants. *A. Rev. Ecol. Syst.* **10**, 173–200.

Harris, H. and Hopkinson, D. A. (1976) *Handbook of Enzyme Electrophoresis in Human Genetics.* Elsevier Publishing Co., New York.

Hillis, D. M. and Moritz, G. (1990) *Molecular Systematics.* Sinauer Associates Inc., Massachusetts.

Kephart, S. R. (1990) Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. *Am. J. Bot.* **77**, 693–712.

Koenig, R. and Gepts, P. (1989) Allozyme diversity in wild *Phaseolus vulgaris*: further evidence for two major centers of genetic diversity. *Theoret. Appl. Genet.* **78**, 809–817.

Levin, D. A. (1978) Some genetic consequences of being a plant. In *Ecological Genetics: The Interface* (Brussard, P. F., ed.), pp. 189–212. Springer-Verlag, New York.

Lewontin, R. C. (1974) *The Genetic Basis of Evolutionary Change.* Columbia University Press, New York.

Liston, A. (1992) Isozyme systematics of *Astragalus* sect. *Leptocarpi* subsect. *Californici* (Fabaceae). *Syst. Bot.* **17**, 367–379.

Loveless, M. D. and Hamrick, J. L. (1984) Ecological determinants of genetic structure in plant populations. *A. Rev. Ecol. Syst.* **15**, 65–95.

Markert, C. L. and Faulhaber, I. (1965) Lactate dehydrogenase isozyme patterns of fish. *J. Exp. Zool.* **159**, 319–332.

Pasquet, R. S. (1993) Variation at isozyme loci in wild *Vigna unguiculata* (Fabaceae, Phaseoleae). *Pl. Sys. Evol.* **186**, 157–173.

Poulik, M. D. (1957) Starch gel electrophoresis in a discontinuous system of buffers. *Nature* **180**, 1477–1479.

Ridgway, G., Sherburne, S. W. and Lewis, R. D. (1970) Polymorphism in the esterases of Atlantic herring. *Trans. Am. Fish. Soc.* **99**, 147–151.

Shaklee, J. B., Allendorf, F. W., Moritz, D. C. and Whitt, G. S. (1990) Gene nomenclature for protein-coding loci in fish. *Trans. Am. Fish. Soc.* **119**, 2–15.

Schinkel, C. and Gepts, P. (1989) Allozyme variability in the Tepary Bean, *Phaseolus acutifolius* A. Gray. *Pl. Breed.* **102**, 182–195.

Small, E., Warwick, S. I. and Brookes, B. (1992) Isozyme variation and alleged progenitor-derivative relationships in the *Medicago murex* complex (Fabaceae). *Pl. Syst. Evol.* **181**, 33–43.

Soltis, D. E. and Soltis, P. S. (1989) *Isozymes in Plant Biology.* Chapman and Hall, London.

Soltis, D. E., Haufler, D. C., Darrow, D. C. and Gastony, G. J. (1983) Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Am. Fern J.* **73**, 9–27.

Swofford, D. L. and Selander, R. B. (1981) *BIOSYS-1*: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* **72**, 281–283.

Van der Bank, F. H. (1995) Allozyme variation in a freshwater mussel population (*Coelatura kunenensis* Mousson 1887) from southern Africa. *Water SA* (in press).

Van Wyk, B.-E. (1983) 'n Monografiese studie van die genus *Virgilia* Poir. M.Sc. thesis, University of Stellenbosch. [In Afrikaans.]

Van Wyk, B.-E. (1986) A revision of the genus *Virgilia* (Fabaceae). *S. Afr. J. Bot.* **52**, 347–353.

Van Wyk, B.-E. and Winter, P. J. D. (1994) Chemotaxonomic value of anthocyanins in *Podalyria* and *Virgilia* (Tribe Podalyrieae: Fabaceae) **22**, 813–818.

Veen, G., Greinwald, R., Witte, L., Wray, V. and Czygan, F.-C. (1991) Alkaloids of *Virgilia divaricata* and *V. oroboides*. *Phytochemistry* **30**, 1891–1895.

Ward, R. D. (1977) Relationships between enzyme heterozygosity and quaternary structure. *Biochem. Genetics* **15**, 123–135.

Whitt, G. S. (1970) Developmental genetics of the lactate dehydrogenase isozymes of fish. *J. Exp. Zool.* **175**, 1–35.