

New Genetic Approaches to Plant Conservation

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INTRODUCTION

The horticultural and agricultural industries rely on plant genetic resources for the development of new crop varieties. Traditional breeding methods involve making new combinations of the genetic material from each parent with the aim of changing the characteristics from parent to offspring and exploiting the genetic diversity present in the parents. The diversity may include variation in flower colour or disease resistance which can be used to commercial advantage.

We have heard about the role biotechnology can play in the commercial horticulture field, and how new molecular techniques allow genetic material to be introduced in ways not possible using traditional breeding methods. I would like to discuss the ways that new techniques are being used in the field of conservation biology to address issues related to the conservation and management of both native plant communities and individual species. The difference between the use of molecular methods for conservation and the genetic engineering of new crops is that for conservation, the goal is to identify the level of genetic diversity and the population structures of naturally occurring species to devise appropriate management strategies rather than the introduction or manipulation of DNA.

Underlying population genetic analysis is the assumption that genetic variation is beneficial for most species because it allows populations to adapt to changing conditions (Barrett and Kohn, 1991). The more genetic diversity which is present in a species, the more choice there is in selecting individuals with desirable traits, e.g. lack of diversity in crop plants can make them susceptible to disease. The same principle applies to natural populations.

The application of population genetics to conservation issues can be useful in defining units for conservation and also for setting priorities for management and the conservation of genetic resources (Avisé, 1994; Moritz, 1994; Volger and deSalle, 1994). Genetic information can be used to decide the size and design of reserves or what methods of sampling and replanting to apply to specific revegetation or rehabilitation sites.

THE TECHNIQUES

Molecular techniques can be divided into those that look: (1) indirectly at variation in particular genes by studying gene products (isozymes or allozymes) or (2) directly at differences in DNA structure (direct sequencing, differences in fragment lengths). Molecular techniques require only small amounts of material so that samples of rare or endangered species can be taken without being detrimental to individual plants. Sophisticated methods of defining genetic variation are usually not applicable to conservation issues for a number of reasons:

- 1) Often, little is known of the breeding systems and reproductive biology of species which are not commercially exploited.

- 2) Funding is scarce for research in areas which do not have the potential for commercially viable outcomes.

Table 1. Advantages and disadvantages of some techniques used for genetic analysis.

Techniques	Advantages	Disadvantages
Proteins	Allozymes	
	Simple relationship between gene and gene product (enzyme)	Only 20-30% of base substitutions in the gene result in detectable change in the protein using standard electrophoretic conditions.
	Codominant expression so heterozygotes can be detected.	Only a small percentage of the genome can be sampled using protein electrophoresis.
	Mendelian inheritance.	Modification of isozymes within the cell can result in patterns with low resolution which are difficult to interpret.
	Consistent location within cells.	
	Known biochemistry and structure of enzymes.	
	Similarity of apparently homologous isozyme loci and their allozyme patterns between different species.	
DNA	RAPDs	
	No previous knowledge of the targeted genome is required.	Each marker is considered a dominant locus (can only be present or absent) so it is not possible to distinguish between homozygotes and heterozygotes.
	Only a small amount of DNA (10 to 30 ng) is required for each reaction.	Technique is very sensitive to reaction conditions and banding patterns can differ from lab to lab.
	Relatively quick, simple and inexpensive compared to other techniques for genetic analysis.	Detects variation at random with no distinction between coding and non-coding regions.

Table 1. continued

	Markers inherited in mendelian fashion	
	DNA preparation does not need to be as clean as other DNA techniques.	
RFLPs	Detects reliable molecular markers.	Requires previous knowledge of plant genome. Requires "clean" DNA. Relatively expensive and time-consuming.
DNA Sequencing	Good for comparison of specific regions. Suitable for phylogenetic analyses.	Requires previous knowledge of plant genome. Requires "clean" DNA. Time-consuming and relatively expensive.
Microsatellites	Can detect variation which is overlooked by other molecular methods.	Development requires expensive work initially. Not cost-effective unless the species will be the subject of detailed analyses.

3) Broad information over the species as a whole is more valuable than detailed genetic information on a small number of individuals.

ADVANTAGES AND LIMITATIONS

The different analytical methods have their own advantages and limitations (Table 1) Details are given by various authors (Hedrick and Miller, 1992; Misretta 1994).

Isoenzymes. Isozyme analysis has been used for population studies since the 1960s. It enables different forms of the same enzyme to be visualised on an electrophoretic gel because of differences in electrophoretic mobility. It remains a valuable tool and often complements more recently developed molecular methods.

DNA Analyses. Marker bands can be generated by cutting DNA with restriction enzymes to detect differences in the length of DNA fragments (RFLPs) or by PCR amplification of a stretch of DNA between specific sites to which short single-stranded primers attach and serve as starting and end points for a PCR. Molecular techniques generally survey DNA differences at random rather than loci which are adaptively important. Practical applications for molecular genetic analysis are given below.

PRACTICAL APPLICATIONS

Grevillea scapigera is a prostrate woody shrub which is close to extinction. It occurs in the wheatbelt of south western Australia where the isolated remnants occur within a 50 km radius. A genetic study using both isozymes and RAPDs was undertaken (Rossetto et al., 1995) to help select individuals which could be used in reintroduction trials as part of the species recovery program. It was found that the genetic variation was evenly spread throughout individuals in the remaining populations suggesting that the sites had become fragmented only recently from a more continuous distribution. It was recommended that plants from all populations could be used to create new populations without any detrimental effects.

Astelia australiana is a rare perennial lily endemic to some rainforest regions of Victoria. Like *G. scapigera*, it has also become endangered due to habitat loss. It grows up to 2 m tall with strap-like leaves which arise from tufts interconnected by rhizomes. The interconnections rot away over time making it impossible to determine whether individual tufts originated from a single genotype. Genetic analysis using RAPDs was used to see if the different sites were comprised of genetically different plants or single clones and whether a disjunct site at the Otway Ranges was a natural occurrence or the result of human-mediated transport. Genetic analysis showed that the diversity was not spread evenly over all the populations and that the Otways site was a natural occurrence. It was recommended that emphasis should be placed on conserving as many populations as possible including the Otways site to maximise the genetic diversity in the species.

Agrostis adamsonii is a rare Victorian grass which occurs in saline depressions within the western plains grasslands. It was not recorded between 1854 and 1985. It is similar to other native *Agrostis* species which grow nearby and it is suspected that it has always been rare and has been overlooked in the past. Genetic analysis in conjunction with a study on the breeding system will enable a conservation plan to be developed based on what can be deduced about the history of the species.

Poa faucettiae is an alpine grass which is found in the Australian Capital Territory and Victoria. It is already used for revegetation. Seed is collected from alpine sites, grown at a lower altitude and the resulting seed is used to provide seedlings for planting out. Genetic analysis is to be used to compare the different populations. If they are similar, seedlings from any population can be used for revegetation in the alpine region. However, if there are marked differences between populations, then only seedlings from nearby areas will be used in revegetation programs.

Adriana quadripartita is a species belonging to the family Euphorbiaceae. It is distinguished from another species, *A. klotzschii*, by its glabrous leaves and fruit. Sometimes the plants are found growing together and it is suspected that they may be variable forms of the same species. By comparing the genetic variation between the two species growing separately and together, in conjunction with taxonomic characters, it should be possible to decide whether to retain the two species or consider them as one highly variable species. The implications for management change because if two species are recognised then *A. quadripartita* is protected under the Victorian Flora and Fauna Guarantee Act (1988) but the more common *A. klotzschii* is not. If only a single species is recognised the name *A. quadripartita* has precedence and so both the glabrous and pubescent taxa would be protected in Victoria.

LITERATURE CITED

- Avise, J.C.** 1994. Molecular markers, natural history and evolution. Chapman and Hall, London.
- Barrett, S.C.H and J.R. Kohn.** 1991. Genetic and evolutionary consequences of small population size in plants: Implications for conservation, p. 3-30. In: D.A. Falk and K.E. Holsinger (eds.). Genetics and conservation of rare plants. Oxford Uni Press, London.
- Hedrick, P.W. and P.S. Miller.** 1992. Conservation genetics: Techniques and fundamentals. *Ecol. Appl.* 2:30-46.
- Misretta, O.** 1994. Genetics of species reintroductions: Applications of genetic analysis. *Biod. Conserv.* 3:184-190.
- Moritz, C.** 1994. Defining evolutionary significant units for conservation. *TREE* 9:373-375.
- Rossetto, M., P.K. Weaver, and K.W. Dixon.** 1995. Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera* (Proteaceae). *Mol. Ecol.* 4:321-329.
- Volger, A.P. and R. deSalle.** 1994. Diagnosing units of conservation management. *Cons. Biol.* 8:354-363.