

Chapter

5

Using Genetic Data to Help Guide Decisions about Sampling



Michael F. Fay

Jodrell Laboratory, Royal Botanic Gardens, Kew,
Richmond, Surrey TW9 3DS, UK

Summary

Many new genetic fingerprinting techniques were developed in the 1990s, and some of these are now being increasingly used to study genetic variation in populations of wild plant species. Examples are presented of the use of AFLP (amplified fragment length polymorphism) and plastid microsatellites in the characterisation of genetic variation in wild species. How this information can be used to inform sampling strategies for seed banking is discussed.

Introduction

In the late 20th century, molecular biology underwent rapid development, and following this, many new genetic techniques have appeared. The development of techniques including DNA sequencing and various forms of genetic fingerprinting have transformed the field of genetics from a largely descriptive to a high technology-based cutting edge science. Many areas of study including medicine, forensics and population genetics have changed greatly as a result. For conservation biology, the ability to use only small quantities of DNA derived from small original quantities of plant tissue (e.g., a small piece of leaf tissue, a single seed or even a single pollinium or pollen grain; Fay *et al.*, 2000; Fay and Salazar, 2001; Pedersen *et al.*, 1996; Widmer *et al.*, 2000) in techniques based on the polymerase chain reaction (PCR; Saiki *et al.*, 1988) has opened up major new possibilities. Before the development of PCR, the amount of plant tissue required was far greater and this meant that the techniques available were not generally applicable for use with rare and endangered species.

Techniques available for the assessment of genetic diversity range from quantification of morphological variation (including morphometrics and cladistic analysis) to protein (mostly alloenzymes) and DNA-based techniques, often collectively referred to as molecular techniques. The range of techniques has been reviewed and discussed by various authors (e.g., Schaal *et al.*, 1991; Weising *et al.*, 1995; Chase and Fay, 1997; Qamaruz-Zaman *et al.*, 1998; Fay and Krauss, in press) and will not be reviewed in detail here. Rather, the application of some of these new techniques to genetic studies of populations of wild plant species will be discussed, with particular reference to how they might inform conservation practice. This area has great significance for seed banking. Many species held in seed bank are only represented by seeds collected from one or a few populations, and without prior knowledge of the distribution of genetic variation within and between populations, it is possible that significant populations may not be included in the sampling. In

an ideal world, genetic screening of all species prior to seed banking would be desirable. Clearly this is not feasible, due to funding and time limitations, but the techniques now available provide the opportunity to undertake studies on some of the rarest and most endangered species. These are the species for which this type of information is the most critical.

AFLP™ (Vos *et al.*, 1995) is now widely used in conservation-related studies. It has the advantages of being a highly reproducible technique (Mathes *et al.*, 1998) producing multilocus genetic fingerprints from across the nuclear genome. For these types of studies, this makes AFLP desirable when compared to random amplified polymorphic DNAs (RAPDs; Williams *et al.*, 1990) and microsatellites which provide information on a single locus. AFLP also has the advantage of a much shorter development time than microsatellites. For these reasons, AFLP have become the ‘tool of choice’ for projects looking at within- and between-population variation in wild plant species, and it has been used with a wide variety of taxa including *Astragalus* (Travis *et al.*, 1996), *Populus* (Winfield *et al.*, 1998, Fay *et al.*, 1999), *Phyllis* (Richardson, 1999; Richardson *et al.*, submitted), *Pedicularis* (Schmidt and Jensen, 2000), *Medusagyne* (Fay *et al.*, 2000), *Rothmannia* (Fay *et al.*, 2000) and *Dactylorhiza* (Hedrén *et al.*, 2001).

With some taxa, however, AFLP can be problematic as a result of large genome size e.g., in *Cypripedium* (Fay and Cowan, 2001) and *Cephalanthera* (Fay *et al.*, 2002), and in these cases it may prove necessary to investigate other marker types. Nuclear microsatellites require a period of development for each new species, but similar regions in the plastid genome can be found and used more readily (Fay and Cowan, 2001).

Case Histories

The use of AFLP and plastid microsatellites and the relevance of the results to seed banking is illustrated with the following case histories which have been chosen to demonstrate how different results affect management decisions.

Tecophilaea cyanocrocus and *Tulipa sprengeri*

Tecophilaea cyanocrocus Leyb. (from Chile; *Tecophilaeaceae*) and *Tulipa sprengeri* Baker (from Turkey; *Liliaceae*) are both bulb species which are extinct in the wild. As part of the background work prior to returning cultivated material to the countries of origin, RBG Kew used AFLP to assess levels of variation in plants from different *ex situ* collections in botanic gardens, etc. (Maunder *et al.*, 2001). Despite their apparently similar history, with both being extensively collected in the wild after their discovery, the results obtained showed

remarkably different genetic structure in the *ex situ* collections. By bringing together material of *T. cyanocrocus* from different collections, the level of genetic variation was dramatically increased relative to that found in the collections held at Kew. With *T. sprengeri*, all material tested from other collections fell within the variation found between plants on the rock garden at Kew. For continued *ex situ* conservation and for repatriation, these results show that different management is necessary. In the case of *T. cyanocrocus*, it appears to be important to conserve plants and seeds from as wide a range of collections as possible, whereas for *T. sprengeri*, a collection of seeds from the plants already at Kew would be sufficient to capture the available variation.

Orchis militaris

The military orchid (*Orchis militaris* L.; *Orchidaceae*) is one of Britain's rarest orchids with only three known populations. Two of these populations (in Buckinghamshire and Suffolk) each contain around 200 plants, but the third (in Oxfordshire) is much smaller, with only six plants being present when the population was discovered. This small population is only 9 km from the Buckinghamshire population and was thought to be derived from this larger population as a result of recent seed dispersal. Before genetic studies were carried out using AFLP and plastid microsatellites (Qamaruz-Zaman, 2000; Qamaruz-Zaman *et al.*, 2002), this population was thus not regarded as being of great significance. However, the genetic data have shown this to be wrong. AFLP demonstrates clearly that the three populations are distinct from each other, and there is no evidence that the Oxfordshire population is derived from the Buckinghamshire population. In addition, the Suffolk population, despite its size, has remarkably little genetic variation, whereas in the Oxfordshire population each of the original plants is clearly distinguishable. Thus, a small sample of seed from Suffolk will capture all the genetic diversity from that population, whereas much more thorough sampling is necessary at the other two sites. Plastid microsatellites also demonstrated the distinctiveness of the Oxfordshire population (Qamaruz-Zaman *et al.*, 2002).

Cypripedium calceolus

The lady's slipper orchid (*Cypripedium calceolus* L.; *Orchidaceae*) is in an even more parlous state than *O. militaris*, with only one plant being known in the wild in the UK. In addition to the wild plant, there are several plants of known wild source in collections and two putatively introduced specimens growing in semi-natural habitat. AFLP proved to be highly problematic in this species (Fay and Cowan, 2001) due to the very large genome size (approximately 180 times that found in *Arabidopsis thaliana* (L.) Heynh.; Bennett *et al.*, 2000), and five plastid microsatellite regions for use with this species have been developed by RBG Kew. The one remaining wild plant and all the plants of known wild origin in the UK possess one or other of the two common Western European fingerprints, but the two putatively introduced plants had fingerprints not

otherwise found in Western European material. One matched material from Austria, but the other does not match any material tested. DNA sequence data provide an explanation for this situation, as these show this plant to be a representative of the morphologically similar North American *C. parviflorum* Salisb. complex! On the basis of these data, both these plants are being excluded from further propagation exercises (Fay *et al.*, 2003).

Conclusions

These examples demonstrate that decisions taken without genetic data can be misleading, and for some of these species, significant genetic variation would have been missed if the genetic data were not taken into account. When possible, screening of populations should be undertaken so that informed decisions can be made about *in situ* and *ex situ* conservation, particularly for high priority species.

Acknowledgements

Much of the work discussed here would not have been possible without funding from the Friends of Kew Threatened Plants Appeal for *Tecophilaea cyanocrocus* and *Tulipa sprengeri*, English Nature (as part of the Species Recovery Programme) for *Cypripedium calceolus* and *Orchis militaris*, and Universiti Putra Malaysia for *O. militaris* (as part of Faridah Qamaruz-Zaman's PhD). Robyn S. Cowan is also thanked for her continued support with the laboratory work and data analysis.

References

- Bennett, M.D., Bhandol, P. & Leitch, I.J. (2000). Nuclear DNA amounts in angiosperms and their modern uses – 807 new estimates. *Annals of Botany* **86**: 859–909.
- Chase, M.W. and Fay, M.F. (1997). Molecular tools for conservation genetics: an update on recent methods, pp. 155–167. In: D.H. Touchell and K.W. Dixon (eds). *Conservation into the 21st century*. Kings Park and Botanic Gardens, West Perth, Australia.
- Ellstrand, N.C. and Elam, D.R. (1993). Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* **24**: 217–242.
- Falk, D.A. and Holsinger, K.E. (eds.) (1991). *Genetics and conservation of rare plants*. Oxford University Press, Oxford, UK.
- Fay, M.F., Bone, R., Cook, P. and Maurin, O. (2003). *Genetic characterisation of Cypripedium calceolus (Orchidaceae) using plastid DNA length polymorphisms: biogeographic patterns and identification of introduced plants*. Report to English Nature. Royal Botanic Gardens, Kew, UK.
- Fay, M.F. and Cowan, R.S. (2001). Plastid microsatellites in *Cypripedium calceolus (Orchidaceae)*: genetic fingerprints from herbarium specimens. *Lindleyana* **16**: 151–156.
- Fay, M.F. and Krauss, S.L. (in press). Orchid conservation genetics in the molecular age, pp. 91–112. In: K.W. Dixon, S.P. Kell, R.L. Barrett and P.J. Cribb (eds). *Orchid conservation*. Natural History Publications, Kota Kinabalu, Sabah.
- Fay, M.F. and Salazar, G.A. (2001). *Using DNA sequencing to confirm the identity of pollinia – a case study with Orchis militaris*. Report to Berks, Bucks and Oxon Wildlife Trust and English Nature. Royal Botanic Gardens, Kew, UK.
- Fay, M.F., Cowan, R.S., Beltran, G. and Allen, B. (2000). Genetic fingerprinting of two endemics from the Seychelles – *Medusagyne oppositifolia (Medusagynaceae)* and *Rothmannia annae (Rubiaceae)*. *Phelsuma* **8**: 11–22.
- Fay, M.F., Lledó, M.D., Kornblum, M.M. and Crespo, M.B. (1999). From the waters of Babylon? *Populus euphratica* in Spain is clonal and probably introduced. *Biodiversity and Conservation* **8**: 769–778.
- Fay, M.F., Qamaruz-Zaman, F., Cowan, R.S. and Thornton, H. (2002). Molecular techniques for orchid conservation genetic studies, pp. 152–157. In: J. Clark, W. M. Elliott, G. Tingley and J. Biro (eds). *Proceedings of the 16th World Orchid Conference*. Vancouver Orchid Society, Vancouver, Canada.
- Frankham, R. (1995). Conservation genetics. *Annual Review of Genetics* **29**: 3305–327.
- Matthes, M.C., Daly, A. and Edwards, K.J. (1998). Amplified fragment length polymorphism (AFLP), pp. 183–190. In: A. Karp, P.G. Isaac & D.S. Ingram (eds). *Molecular tools for screening biodiversity. Plants and animals*. Chapman & Hall, London, UK.
- Maunder, M., Cowan, R.S., Stranc, P. and Fay, M. F. (2001). The genetic status and conservation management of two cultivated bulb species extinct in the wild: *Tecophilaea cyanocrocus* (Chile) and *Tulipa sprengeri* (Turkey). *Conservation Genetics* **2**: 193–201.

- Petersen, G., Johansen, B., and Seberg, O. (1996). PCR and sequencing from a single pollen grain. *Plant Molecular Biology* **31**: 189–191.
- Qamaruz-Zaman, F. (2000). *Conservation genetics of rare and endangered British orchids*. PhD thesis. University of Cambridge, Cambridge, UK.
- Qamaruz-Zaman, F., Chase, M.W., Parker, J.S. and Fay, M.F. (2002). *Genetic fingerprinting studies of *Orchis simia* and *O. militaris**. Royal Botanic Gardens, Kew, UK.
- Qamaruz-Zaman, F., Fay, M.F., Parker, J.S., and Chase, M.W. (1998). Molecular techniques employed in the assessment of genetic diversity: a review focusing on orchid conservation. *Lindleyana* **13**: 259–283.
- Richardson, J.E. (1999). *Molecular systematics of the genus *Phyllica* L. with an emphasis on the island species*. PhD thesis, University of Edinburgh, Edinburgh, UK.
- Richardson, J.E., Fay, M.F., Cronk, Q.C.B. and Chase, M.W. (in press). Species delimitation and the origin of populations in island representatives of *Phyllica* (*Rhamnaceae*). *Evolution*.
- Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Nature (London)* **239**: 487–497.
- Schaal, B.A., Leverich, W.J. and Rogstad, S.H. (1991). A comparison of methods for assessing genetic variation in plant conservation biology, pp. 123–134. In: D.A. Falk and K.E. Holsinger (eds). *Genetics and conservation of rare plants*. Oxford University Press, Oxford, UK.
- Schmidt, K. and Jensen, K. (2000). Genetic structure and AFLP variation of remnant populations in the rare plant *Pedicularis palustris* (*Scrophulariaceae*) and its relation to population size and reproductive components. *American Journal of Botany* **87**: 678–689.
- Travis, S.E., Maschinski, J. and Keim, P. (1996). An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically endangered plant using AFLP markers. *Molecular Ecology* **5**: 735–745.
- Vos, P., Hogers, R., Bleeker, M., Rijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Kuiper, M. and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**: 4407–4414.
- Weising, K., Nybom, H., Wolff, K., and Meyer, W. (1995). *DNA fingerprinting in plants and fungi*. CRC Press, Boca Raton, FL, USA.
- Widmer, A., Cozzolino, S., Pellegrino, G., Soliva, M. and Dafni, A. (2000). Molecular analysis of orchid pollinia and pollinaria-remains found on insects. *Molecular Ecology* **9**: 1911–1914.
- Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**: 6231–6235.
- Winfield, M.O., Arnold, G.M., Cooper, F., Le Ray, M., White, J., Karp, A. and Edwards, K.J. (1998). Study of genetic diversity in *Populus nigra* subsp. *betulifolia* in the Upper Severn area in the UK using AFLP markers. *Molecular Ecology* **7**: 3–10.