Biochemical Systematics and Ecology 58 (2015) 156–161

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Genetic diversity of the endangered *Faucaria tigrina* (Aizoaceae) through ISSR "fingerprinting" using automated fragment detection

Luke Bentley [a], Nigel P. Barker [a, *], Anthony P. Dold [b]

[a] Department of Botany, Rhodes University, Grahamstown 6140, South Africa
[b] Selmar Scholand Herbarium, Department of Botany, Rhodes University, Grahamstown 6140, South Africa

ARTICLE INFO

Article history:
Received 1 October 2014
Accepted 21 November 2014
Available online 5 December 2014

Keywords:
Aizoaceae
Automated detection
Faucaria tigrina
Genetic diversity
ISSR fingerprinting
Conservation genetics

ABSTRACT

Faucaria tigrina (Haw.) Schwantes is a rare, threatened succulent found exclusively on the outskirts of Grahamstown, Eastern Cape, South Africa. There are three extant populations of the species; two large ones (approximately 300 and 620 adults) separated by under two kilometres, and a third very much smaller one slightly further away. Genetic theory expects that smaller, isolated populations face a loss in genetic variation through inbreeding and genetic drift and that as declining genetic variation is linked to a loss in fitness, this species may face extinction in the long term. This study used the ISSR-PCR genetic methods linked to an automated detection platform to determine if these populations are genetically distinct, and whether they are genetically depauperate. Two ISSR primers were used, and the automated detection system identified a total of 572 ISSR loci. An UPGMA clustering analysis showed each population to be genetically distinct, and that the genetic diversity of each population does not appear to be particularly low. *F. tigrina* is separable from the related *Faucaria britteniae* L. Bolus using the ISSR method, suggesting that this method may be appropriate for systematic studies in the Aizoaceae, which comprises many genera with closely related species.

1. Introduction

The process of assessing species (plant and animal) for inclusion in the IUCN Red List of threatened species requires demographic information on the number of individuals, the number of subpopulations (the "population" equating to the species in the IUCN definition), the area or distribution range of these sub-populations, and limited aspects of biology such as generation time (IUCN, 2012). Ideally, knowledge of fluctuations of these variables over time is also informative and aids in the allocation of a species to a threat category. However, the IUCN criteria make no mention of use of data on genetic diversity of candidate species, which is important in light of the generalisation that rare species probably have reduced genetic diversity as a consequence of genetic drift and inbreeding, processes which occur faster in smaller populations (Ellstrand and Elam, 1993; Young et al., 1996). There is some empirical evidence that links small population sizes with reduced genetic diversity. For example, Fischer and Matthies (1998) found a positive correlation between population size and genetic variation

* Corresponding author. Tel.: + 27 46 603 8715; fax: +27 46 603 7355.
E-mail address: n.barker@ru.ac.za (N.P. Barker).

http://dx.doi.org/10.1016/j.bse.2014.11.012
0305-1978/

in *Gentianella germanica*, and genetic variation and gene flow between populations was found to be limited. Buza et al. (2000) measured variation and fixation coefficients in 17 fragmented populations of *Swainsona recta*, the tetraploid pea and found that fixation coefficients correlated with the log of the population size.

It is thus important to maintain populations of rare species above a threshold at which genetic diversity becomes compromised, the "Minimum Viable Population" (MVP). However the minimum size for a MVP is difficult to define. Addressing this question, Traill et al. (2007) undertook a meta-analysis of 141 studies spanning dates from the early 1970s until 2007 and concluded that 4169 individuals is the median number needed in a population for its long-term survival. As populations fall markedly below this number, the population becomes less viable, less fit and more prone to extinction. Lande (1988) stated that, according to empirical examples, demography is more important than population genetics when determining the MVP. However Hogland (2009) considers that the balance between demography and genetics is important. Spielman et al. (2004) presents a meta-analysis assessing if genetic variation was lower in more threatened global populations of plants and animals and concluded that heterozygosity was lower in 77% of the 170 comparisons. Hence it can be concluded that most species are not driven to extinction before the impact of genetic factors (Hoglund, 2009), and that if small populations persist long enough, the negative impacts of reduced genetic diversity will hasten a species demise.

The assessment of genetic diversity of the IUCN-listed species (especially the threatened categories) is important, as these species are likely to have small, genetically isolated sub-populations. The generally accepted statistic or measure of genetic diversity is heterozygosity – a measure that can only be obtained by sampling co-dominant markers; typically allozymes or microsatellites. However, these techniques are costly and can be time consuming. Less rigorous methods to assess genetic diversity generally resort to some sort of DNA "fingerprinting" approach to assess diversity and some measure of polymorphism. Such methods include AFLP (Amplified Fragment Length Polymorphisms), RAPD (Randomly Amplified Polymorphic DNA) and Inter-Simple Sequence Repeats (ISSR). ISSRs have been found to detect higher levels of polymorphism than RFLP (Restriction Fragment Length Polymorphism) and RAPD (Godwin et al., 1997; Potter et al., 2002). Furthermore, they are a relatively cheap and a fast way to analyze genetic variability and individuals which are genetically very similar (e.g. Potter et al., 2002; Sarwat, 2012) and have been successfully used in other studies on rare plants (e.g. Xiao et al., 2004; Chen et al., 2005; Ge et al., 2005; Luan et al., 2006). Many studies that have used ISSRs have employed standard agarose or acrylamide gel electrophoresis combined with ethidium bromide or silver staining methods for visualisation by human eye or computerised band detection packages. However, the use of ISSR primers labelled with fluorescent dyes enable the use of far more sensitive detection methods such as those found in automated sequencing platforms equipped with fragment profiling software. This technology detects many more ISSR fragments, and is able to separate bands differing by a single nucleotide in length. As a result, considerably more data is produced from a single primer (Archibald et al., 2006). This approach has been successfully applied in a number of studies on plant genetic diversity (e.g. Patterson et al., 2009; Paterson and Zachariades, 2013; Taylor et al., 2011; Taylor and Barker, 2012).

Unfortunately, the ISSR method provides data on dominant markers only, and cannot directly be used to estimate heterozygosity. However, formulae that estimate heterozygosity are applicable to this kind of data (Mariette et al., 2002; Nei, 1973). Here we used the ISSR method to assess the genetic diversity of a Red Listed species *Faucaria tigrina*, and comment on the suitability of this method to plant conservation.

Currently listed as vulnerable by the global IUCN list (IUCN, 2012) and endangered in the latest national IUCN Red List (Victor and Dold, 2003; redlist.sanbi.org accessed Sep. 25 2014), *Faucaria tigrina* is a narrow endemic on the outskirts of Grahamstown (Eastern Cape, South Africa). It grows largely in Renosterveld on open rocky patches (Groen and van der Maesen, 1999). Being a narrow endemic, it is vulnerable to large stochastic events which could affect the population size. Previously known from a number of populations, it is now confirmed at only three sites, despite intensive searches by the authors at two other sites where it was previously reported. It is grown widely in collections by succulent enthusiasts around the world, and there are probably many more plants in cultivation than in the wild. The source of these cultivated plants is unknown, but they must have originated from the Grahamstown area. Given this horticultural and enthusiast interest, the exact localities of the plants studied here are not provided.

2. Materials and methods

2.1. Sampling

Specimens were collected from three populations (sub-populations *sensu* the IUCN) of *Faucaria tigrina* and one population of *Faucaria britteniae* from the Grahamstown vicinity. These populations are all on commonage land and hence under threat from collectors, trampling by livestock, fire, smothering by other vegetation and stochastic climatic conditions such as extended drought. In addition, a proposed housing development poses a threat to one of the populations. As part of ongoing low-level conservation monitoring for this species, populations 1 and 2 have been monitored and censused annually indicating a constancy at about 300 adult plants in population 1 and about 620 for population 2.

Samples for DNA analysis were taken from 37 individuals; 14 from population 1, 16 from population 2, 4 from population 3 and three samples of the closely related *F. britteniae*. The latter samples were included to test the ability of the ISSR method to distinguish between closely related species. Population 3 is very small and the plants are small and appear somewhat unhealthy in appearance (only 4 of the 8 plants found at this site were sampled). For each sample, the top 2 mm of the leaf tip