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Genetic diversity and population structure of the endemic Azorean juniper, *Juniperus brevifolia* (Seub.) Antoine, inferred from SSRs and ISSR markers

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ABSTRACT

Juniperus brevifolia is an important woody species endemic of the Azores archipelago (Portugal), found from coastal to mountain environments. Due to colonization and grazing pressure this species has suffered fragmentation, leading to extinction in one island and being threatened in others. The genetic diversity and population structure of *J. brevifolia* populations was studied to provide guidelines for restoration and conservation programmes. Nuclear Single Sequence Repeats (nSSR) from *Juniperus communis* and *Juniperus przewalskii* and Inter Simple Sequence Repeats (ISSR) were tested and results compared to test the transferability of the microsatellites to *J. brevifolia*. Samples from ten populations over three islands, divided as coastal, mid-altitude and mountain were analysed. Both marker systems revealed results statistically and strongly correlated with each other, and not dependent on population sample size. We observed positive fixation indexes, moderate to high levels of genetic diversity ($h = 0.415$ for nSSR and $h = 0.245$ for ISSR), low to moderate φ_{pt} genetic differentiation (0.070 for nSSR and 0.129 for ISSR) and high gene flow ($N_m > 2.432$). Regarding Nei's genetic distance the coastal communities clustered together reflecting the phenotypic plasticity, but no specific clustering was observed regarding φ_{pt} values. Therefore no populations with substantial genetic differentiation were identified, once the diversity is mostly observed within populations. However it is advised the continuous monitoring of *J. brevifolia*.

1. Introduction

The Azores archipelago, in the North Atlantic Ocean, comprises nine volcanic islands divided into three groups (western group- Flores and Corvo; central group- Terceira, Graciosa, S. Jorge, Pico and Faial; eastern group- S. Miguel and Santa Maria). The endemic vascular flora has about 72 taxa (Silva et al., 2010), most of them a part of the autochthonous "Laurissilva" or laurel forest, which has undergone abusive use by man for over five centuries. Nowadays the laurel forest is represented in small, fragmented patches on the higher lands of S. Miguel, Terceira, Pico and Flores (Fernández-Palacios et al., 2011). A

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substantial reduction of the native forest populations occurred including the key-species *Juniperus brevifolia* (Cedar or Azorean juniper) (Ferreira and Eriksson, 2006; Dias et al., 2007). *Juniperus brevifolia* is considered vulnerable according to the International Union for Conservation of Nature – IUCN (2014) and is mentioned in the Council Directive 2006/105/EC on the conservation of natural habitats and of wild fauna and flora. It is distributed among all islands, except for Graciosa where is extinct and in Santa Maria is facing extinction. Its actual distribution is less than its potential distribution and its continuous decrease will have severe consequences on natural ecosystems, quality and quantity of hydric resources and insular soils, flora and fauna (Dias et al., 2007; Elias and Dias, 2008). Pico preserves the best examples of coastal communities namely in the eastern (Piedade) and north-western parts (Cachorro and Cais do Mourato) (Dias et al., 2007; Elias and Dias, 2008). The widest natural areas of Azorean juniper are located at Terceira and Flores. S. Miguel has suffered a drastic decrease of juniper populations due to high demographic density, introduction of aggressive invasive plant species and intense grazing and livestock activity (Elias and Dias, 2008).

The presence of *J. brevifolia* in the Azores implies one of the most remote juniper colonization known (Rumeu et al., 2011a), representing one of the best examples of endemic species displaying traits favourable for long-distance dispersal and colonization, i.e. dioecy, anemophily, zoochory, multi-seeded cone (Adams, 2011) and mesic habitats requirements (Elias, 2007). The *J. brevifolia* wide distribution is due to its dominancy and co-dominancy at several endemic communities and well adapted morpho-physiology to Azorean ecological conditions (Dias et al., 2007). This species can be divided into three categories: coastal (0–50 m), mid-altitude (500–700 m) and mountain (800–1250 m), and show phenotypic plasticity, where coastal communities show morphological characteristics different from the mountain communities. This may contribute to improve fitness in long-lived tree species (Endler and Mac Lelland. 1988). However, this phenotypic plasticity between coastal and mountain communities raises questions on the possibility of reintroducing this species on islands where populations have declined and on the adequateness of exchanging plant material of multiple origins between islands. Knowledge of the populations' genetic diversity and structure is therefore crucial to develop effective conservation programmes. The combination of a wide geographical range and the presence of dispersed populations make it possible for a plant species to harbour a vast amount of genetic variability. Also the occurrence of peculiar allelic combinations can be exploited in planning conservation strategies (Petit et al., 1998; Meloni et al., 2006).

In a recent study of Silva et al. (2011) it was identified a high degree of differentiation and restricted gene flow between populations, which contradicts the accepted theories for the Azorean plant diversity with low number of radiations and low number of single-island endemics and also contradicts the life traits of this species that favours the long distance dispersal, colonization and gene flow exchange (Carine and Schaefer, 2010; Rumeu et al., 2011a; Schaefer et al., 2011).

Regarding this contradictory information, we studied the genetic diversity and structure of *J. brevifolia* from ten populations of three islands (Pico, S. Miguel and Terceira), by sampling proportional to the populations' size, by using interspecific nuclear simple sequence repeat (nSSR) markers, transferred from *Juniperus communis* and *Juniperus przewalskii* and inter-simple sequence repeats (ISSR), to correlate their results. Both nSSR and ISSR markers are commonly used for measuring genetic diversity. The use of different molecular markers has provided interesting insights into the distribution of genetic diversity among populations and into the evolutionary forces that contributed to the extant structure of diversity. The comparative analysis between different markers is intended to avoid the bias caused by the "marker specific" factors. A significant correlation would indicate congruence between markers and the impact of "marker non-specific" factors (as drift and migration) on levels of diversity. Lack of correlation is likely to reveal that "marker specific" factors (as mutation) are sufficiently different between populations to generate different levels of diversity (Mariette et al., 2001).

Hence, the objectives of this work were: 1) investigate the transferability of nSSR markers among *Juniperus* species and correlation between nSSR and ISSR; and 2) evaluate patterns of genetic diversity and populations' degree of differentiation and genetic structure of *J. brevifolia* within populations, among populations and among islands. These objectives combined will further improve the knowledge on the distribution of *J. brevifolia* and will elucidate the recent contradictory information on this matter.

2. Methods

The authors declare that the experiments within this work comply with the current laws of Portugal, the sampling procedures were authorized and were done in the presence of an Azorean Environmental Conservation Services member, and the laboratory procedures were performed according to the national safety rules.

2.1. Plant material

Young leaves of 300 GPS marked *J. brevifolia* individuals were collected, from ten populations of three islands: Pico (Piedade – Pied, Mistério da Praínha – MPra, Maciço Central – MCen and Cachorro – Cach), S. Miguel (Graminhais – Gram, Lombadas – Lomb and Tronqueiras – Tronq) and Terceira (Serreta/Lagoínha – Serr/Lag, Terra Brava – TBra and Mistérios Negros – MNeg), (Appendix 1). Samples were collected randomly with a minimum of 20 m distance between them. Efforts were made to have the number of individuals collected as proportional as possible to the population's sizes. Individuals were identified as male or female. Plant material was weighted and frozen at −80 °C until further utilization.