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Reduced reproductive fitness of an endemic insular juniper population: An eco-genetic mediation hypothesis

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ABSTRACT

The mutual reinforcement of multiple drivers of global change erodes ecosystem services and accelerates plant population decline worldwide. This is particularly the case for island ecosystems where anthropogenic activity has imperilled insular floras for centuries. Different hypotheses have put forward the combined effects of environmental shifts and genetic factors in driving fecundity decline in threatened populations. In this study, we combined population genetic tools and structural equation models to test the eco-genetic mediation hypothesis, that the environmental conditions influence genetic variation, which in turn affects plant fitness. Our study species is *Juniperus cedrus*, an endangered juniper species endemic to the Canary Islands. Juniper woodlands have been depleted since the arrival of human inhabitants confined extant populations to marginal lands. More recently, long-distance dispersers have been extirpated from the study area, potentially eliminating connectivity among fragmented populations. We expected strong intrapopulation subdivision and high levels of inbreeding and kinship that should negatively affect individual fitness. We found evidence of population subdivision into several mating neighbourhoods ($K = 13$), some of them highly inbred. However, contrary to our expectations, neither inbreeding nor mean kinship mediated a population response to environmental factors. Our results suggest three remedial actions to reverse the declining demographic trends: (i) re-establish native vegetation to ameliorate local environmental conditions; (ii) restore dispersal services to increase connectivity; and (iii) monitor fitness decline to identify lagged effects associated with deforestation. Overall, this study shows that structural equation models combined with population genetic techniques are suitable tools to identify high-order effects among multiple drivers of global change that underlie forest decline.

1. Introduction

Drivers of global change are disrupting ecosystem services causing fast-pace plant population decline worldwide (Dirzo et al., 2014). Conservation biologists have formulated different hypotheses that emphasise the role of positive feedback between environmental, demographic and genetic factors in driving fecundity ("the eco-genetic hypothesis of background extinction", Wiens et al., 2012) and underpinning population extinction (the "extinction vortex", Gilpi and Soulé, 1986). Together, these hypotheses and other derived models posit that extant populations inhabiting degraded habitats are prone to extinction in the long term due to the mutual reinforcement of environmental and genetic factors. Whereas the deleterious effects of increased inbreeding levels are well documented in both plant and animal systems (Frankham, 2005; Lowe et al., 2005) and are significantly enhanced by stochastic dynamics in small populations (Lande, 1988), the complex interaction network of direct and indirect effects among environmental and genetic drivers that operate to trigger fecundity decline in deforested landscapes remains unknown. This is a pressing question because drivers of global change have already caused profound habitat shifts worldwide (Barnosky et al., 2012; Dirzo et al., 2014).

Forest fragmentation is ongoing, and remnant fragments typically occupy marginal lands where suboptimal conditions limit population growth (Cordeiro et al., 2009) and disrupt mating patterns (Breed et al., 2012) and dispersal services (Galetti et al., 2013). Therefore, a large proportion of species of extant forests might undergo some degree of background extinction *sensu* Wiens and Slaton (2012), *i.e.*, a steady and multi-generational reproductive decline that eventually might lead populations to disappear due to the continued loss of suitable habitat. This is particularly the case for remnant populations inhabiting oceanic islands where anthropogenic activity has imperilled insular floras for centuries (Caujapé-Castells et al., 2010).

In this study, we combine population genetic tools and structural equation population models (Grace et al., 2012) to test the mediation hypothesis that sub-optimal environmental conditions cause fitness decline through genetic factors, namely increased inbreeding and kinship

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levels. Our study species is an endangered juniper endemic to the Canary Islands, *Juniperus cedrus* Webb & Berth. This species has been subjected to severe habitat loss since the human occupation of the Canary archipelago (first millennium BC, del Arco et al., 1997), due to intensive felling, herbivory by introduced mammals, and increasingly recurrent human-driven fires (Parsons, 1981). Moreover, its only long-distance seed dispersal vector (*Corvus corax*) has been eradicated from part of its distribution range. As a result of these disturbances, *J. cedrus* is currently confined to craggy inaccessible sites with suboptimal environmental conditions (Nogales et al., 2014).

In a previous study, we recorded reduced reproductive fitness in a remnant population of *J. cedrus* (Rumeu et al., 2009). In this study, however, a mediation hypothesis is specifically formulated, proposing that local conditions near adult trees (*i.e.*, the ecological neighbourhood) influence individual genetic inbreeding and local kinship, which in turn might trigger a decline in reproductive fitness. To that end, we first searched for evidence of inbreeding based on fixation indices (F-statistics) and Bayesian genetic clustering methods (Guillot et al., 2009). If environmental factors disrupt mating patterns, for example due to low density of reproductive adults, the study population would be expected to consist of a set of subdivided demes (mating neighbourhoods) rather than a panmictic population (Pannell and Fields, 2014). Second, we characterised the ecological neighbourhood around each tree and measured, on an individual basis, phenotypic and genetic factors that we hypothesised to influence reproductive fitness. Third, we formulated our hypothesis in terms of two structural equation models that accommodated direct and indirect effects of environmental, genetic and phenotypic traits. Finally, we discuss the importance of our results for establishing evidence-based management plans. All in all, this study illustrates that the mediation hypothesis poses a suitable framework to investigate complex causal relationships and high-order effects between multiple environmental, phenotypic, and genetic factors underpinning rampant population declining trends.

2. Materials and methods

2.1. Study population

We conducted our research on the island of Tenerife where, based on historical records, some authors consider that juniper forests might have formed a vegetation belt in the timberline area, which has its lower altitudinal optimum situated at the Canary pine forest treeline, *ca.* 1500–2000 m above sea level (a.s.l) (Höllermann, 1978). Our study population was at *Riscos de La Fortaleza* (Parque Nacional del Teide, 28°19′N, 16°36′W), a craggy rock formation located at *ca.* 2100 m a.s.l. that harbours one of the best remnant populations of *J. cedrus* ($N = ca.\ 200$) (Fig. 1). It is influenced by a typical high-mountain climate with a mean annual temperature of 10.7 °C, and maximum temperatures that range between 9.7 °C and 16.4 °C and minimum temperatures that vary between −0.2 °C and 12.6 °C throughout the year. This population receives an annual precipitation of approximately 365.5 mm, most of which falls during the winter months (Bustos and Delgado, 2004). *Riscos de La Fortaleza* is an escarpment where individuals grow in a scattered formation both on the upper plateau and the lower slopes (Fig. 1).

The Canarian juniper is a dioecious, anemophilous and endozoochorous taxon. According to several studies carried out on the population of *Riscos de La Fortaleza*, fleshy female cones contain 2.41 ± 0.69 seeds per cone on average, and viable seeds are significantly heavier than non-viable ones (Rumeu et al., 2009). *C. corax*, the only large-bodied seed disperser, became locally extinct *ca.* 20 years ago in the range of this population (Martín and Lorenzo, 2001). This resulted in the loss of a highly effective long-distance seed disperser whose gut treatment enhances seed germination at distant sites from the mother tree (Nogales et al., 1999; Rumeu et al., 2011). Therefore, only small- to medium-sized vertebrates such as lizards (*Gallotia galloti*) and wintering ring-ouzels (*Turdus torquatus*) serve as seed dispersers in the area (Rumeu et al., 2011). Secondary seed dispersal by kestrels (*Falco tinnunculus*) preying on lizards has also been documented to occur occasionally (Padilla et al., 2012). Thus, given the more limited movement range of the lizards, only the migrant thrush *T. torquatus* provides regular medium-distance dispersal services for this endangered juniper, probably <100 m (Morales et al. (2013). Additionally, the endemic finch *Fringilla teydea* has been observed preying on *J. cedrus* seeds during the winter months (Rumeu et al., 2011).

2.2. Sample collection and genotyping procedure

Leaf tissue from all accessible individuals in the population was collected and dry-stored awaiting DNA extraction (~95% of the total population). These samples comprised 195 individuals: 102 sexually mature trees (47 females, 54 males and one monoecious individual) and 93 sexually immature trees (Fig.1). We mapped all sampled individuals with a GPS receiver Garmin Dakota 10 (GARMIN Ltd., Canton of Schaffhaunsen, Switzerland) that provides the position of each tree with 2–5 m horizontal accuracy, and isolated genomic DNA from silica-gel dried needles using the Qiagen DNeasy mini-kit (QIAGEN, Hilden, Germany). Individuals were genotyped with 14 polymorphic microsatellite markers, 13 specifically designed for *J. cedrus* (Jce01–13, Rumeu et al., 2013) and one previously tested for *J. communis* (Michalczyk et al., 2006). See Rumeu et al. (2013) and Michalczyk et al. (2006) details on genotyping protocols. Amplified fragments were analysed using an ABI 3730 DNA analyser (Secugen S.L., Madrid, Spain) and scored manually and independently by two people using *GeneMapper* 4.0. Scores were cross-checked to minimise genotyping errors. We tested for the presence of allelic dropout and null alleles in a subset of 28 samples. No evidence of stuttering or allele dropout was detected based on analyses performed with Micro-checker (Oostherhout et al., 2004) but three markers (Jce08, Jce09, Jce10) showed a heterozygosity deficit, which suggests the presence of null alleles.

2.3. Fitness variation

We ruled out counting seeds per cone as a measure of female fitness because juniper trees tend to produce a high number of fully developed but empty seeds (Chambers et al., 1999). Crop size is highly variable among juniper individuals, so it only works as a reliable proxy of reproductive output when data are averaged across several years (Chambers et al., 1999). We lacked multi-year data on crop size and since viable seeds are reported to be significantly heavier than non-viable seeds (Rumeu et al., 2009) we used mean seed weight as a reliable estimate of plant fitness, for which we had previous evidence of intrapopulation variation (Rumeu et al., 2009). We are aware that any proxy to quantify fecundity variation would fail to capture some aspects of the reproductive biology of this species. Yet, we are confident that mean seed weight is a suitable proxy to test the eco-genetic mediation hypothesis because: (*i*) there is previous literature that shows that this variable is sensitive to environmental heterogeneity; and (*ii*) the applied structural equation models include phenotypic traits (such as basal stem circumference) and their effect on mean seed weight, if any, can be quantified. To assess mean seed weight on an individual basis, *ca.* 100 seed cones per plant were collected in order to test for fitness variation among females (only 20–40 seed cones from four individuals who produced an extremely low crop were collected). Four female trees with no seed cones and one unreachable individual were excluded from our sample. The population at *Riscos de La Fortaleza* was partially burned by a fire in 2007 that affected 9 individuals in our sample. We retained mean seed weight data previously reported by Rumeu et al. (2009) for three out of the nine female trees partially burnt in 2007. To obtain mean seed weight per female tree, all collected cones were opened and pulp remnants were carefully removed from the seeds before weighing *ca.* 100 seeds per plant whenever possible. We then evaluated whether mean