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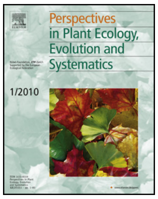

Research article

Narrow endemics to Mediterranean islands: Moderate genetic diversity but narrow climatic niche of the ancient, critically endangered *Naufraga* (Apiaceae)

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ABSTRACT

Narrow endemics constitute the cornerstone of Mediterranean plant diversity. *Naufraga balearica* (Apiaceae) is a critically endangered, extremely narrow endemic plant from the western Mediterranean island of Majorca. Because the species belongs to a monotypic genus, *N. balearica* was hypothesized to be a palaeoendemism. Here we conducted phylogenetic dating, population genetic and climatic niche analyses in order to understand the evolutionary history and conservation perspectives of this flagship species. Phylogenetic dating analysis of nuclear and plastid DNA sequences revealed a late Miocene to early Pliocene divergence between *Naufraga* and its sister genus *Apium*, supporting the palaeoendemic status of the former. Amplified fragment length polymorphism (AFLP) markers and plastid DNA sequences of the five *Naufraga* populations revealed moderate genetic diversity. This diversity is in line with that of other palaeoendemisms from western Mediterranean islands, as revealed by a comparison with 22 other narrow endemic species from this region. Despite the fact that all *Naufraga* populations are located at a maximum distance of 10 km in a straight line, a strikingly strong population differentiation was found for AFLP markers, which is explained by long-term isolation likely related to short-range pollination and dispersal strategies of the species. While the species is not genetically impoverished, species distribution modelling and microclimatic monitoring revealed that narrow ecological requirements underlie the current extreme rarity of *Naufraga* and may jeopardize its long-term survival. Our results indicate that a multidisciplinary approach provides powerful tools to develop conservation strategies for evolutionarily singular lineages.

Introduction

The Mediterranean Basin, with c. 25,000 plant species (Quézel, 1985), constitutes one of the world's major biodiversity hotspots (Myers et al., 2000). Around 60% of plant species endemic to the Mediterranean region are narrow endemics, i.e. species whose distribution is restricted to a single, small area (Thompson, 2005). Hence, narrow endemics (both palaeo- and neoendemics) are considered to constitute the cornerstone of Mediterranean plant diversity (Thompson, 2005). Some of these species, termed 'extremely narrow endemics' (ENEs), are known from one or very few populations (≤5) and display very small census sizes (≤500 individuals) (López-Pujol et al., 2013). ENEs are of particular conservation concern because of the high extinction risk associated to rarity (O'Grady et al., 2004). In addition, ENEs usually display low levels of genetic diversity (Gitzendanner and Soltis, 2000; López-Pujol et al., 2013), which may limit their evolutionary viability. This genetic impoverishment is variously viewed as either a cause or consequence of rarity (Gitzendanner and Soltis, 2000). Indeed, the causes of species rarity have long been discussed, and a combination of ecological, historical and genetic factors is generally invoked to account for it (Kruckeberg and Rabinowitz, 1985).

Naufraga balearica (Fig. 1A) is an extremely narrow endemic plant from the northern coast of the western Mediterranean island

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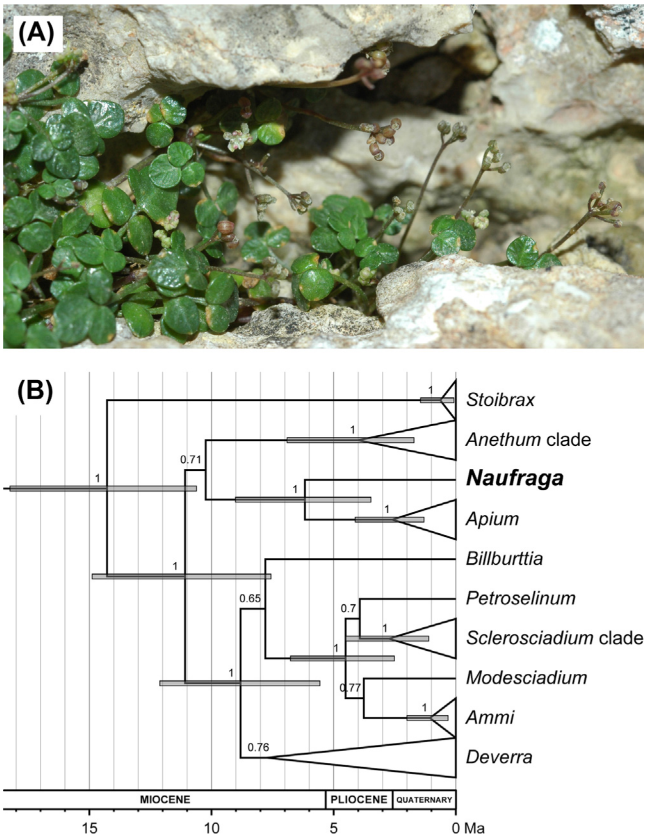

Fig. 1. (A) Specimens of *Naufraga balearica* with flowers and fruits, Cap de Catalunya population (photograph by P. Vargas). (B) Phylogenetic dating analysis of the tribe Apieae based on combined nuclear (ITS) and plastid (*rps*16) DNA sequences. The maximum clade credibility tree produced by a relaxed molecular-clock analysis in BEAST is shown. Outgroup taxa have been pruned for clarity. Values above branches indicate Bayesian posterior probabilities (PP). Node bars represent the 95% highest posterior density intervals for the divergence time estimates of clades with PP = 1.

of Majorca (Balearic Islands, Spain) (Rosselló, 2010). It is listed as critically endangered (CR) in the IUCN Red List (Moreno, 2011) and the Red List of Spanish vascular flora (Moreno, 2008). The species was first described from a single locality (Coves Blanques) discovered in 1962 (Constance and Cannon, 1967; Duvigneaud, 1970). Additional populations were found decades later in the Formentor Peninsula, not far from the *locus classicus* (Bibiloni and Soler, 2002). One locality (Finucchiaghia) was also reported in Corsica in 1981, but became extinct shortly after (Gamisans et al., 1996; Fridlender, 2001). Genetic analysis of cultivated Corsican plants indicated a very close relationship with the Coves Blanques population (but not with other populations from Majorca), which casted doubt on the spontaneity of *N. balearica* in Corsica (Fridlender and Boisselier-Dubayle, 2000).

It has long been hypothesized that *N. balearica* is a palaeoendemism (Duvigneaud, 1970). Palaeoendemics have historically been defined as relics of earlier floras which have survived in a limited portion of their past territory (Wulff, 1943; Favarger and Contandriopoulos, 1961). They are systematically isolated as a result of their early divergence, and they did not necessarily originate in the area they currently occupy. On the contrary, neoendemics originated recently in a given region and have not yet spread beyond it. They are closely related to other species, frequently in the same region. Phylogenetic analyses based on nuclear internal transcribed spacer (ITS) sequences have revealed that *Naufraga* is sister to the genus *Apium* (c. 20 spp.), and divergence between the two genera has been dated back to >4.8 Ma (Spalik et al., 2010; Banasiak et al., 2013). These results are congruent with a palaeoendemic status of *Naufraga*. However, additional evidence (from phylogenetics, population genetics and ecology) is required before firm conclusions can be drawn.

A previous population genetic analysis based on random amplified polymorphic DNA (RAPD) markers found strong genetic differentiation between populations of *N. balearica* (Fridlender and Boisselier-Dubayle, 2000). However, the population sampling in that study was unsatisfactory (number of localities included) and the reliability of RAPD markers has been questioned because of their low reproducibility (Newton et al., 1999). More reliable markers, together with a deeper sampling of individuals and populations, are therefore needed for a reliable genetic characterization of *N. balearica* populations.

Here, phylogenetic and population genetic analyses (based on nuclear and plastid DNA markers) and species distribution modelling were conducted to achieve the following objectives: (1) to estimate the divergence time of *N. balearica*; (2) to disclose the genetic diversity and spatial genetic structure of extant populations; and (3) to characterize the climatic niche of the species both at the macro- and microclimatic scales. Our results were further integrated with previous ecological and life history data in order to understand the evolutionary history and conservation perspectives of the species. Our working hypothesis in this study was that the current rarity of *N. balearica* can be explained by its narrow ecological requirements, together with its short-range pollination and dispersal strategies.

Materials and methods

Study species

Naufraga balearica Constance & Cannon (Apiaceae, Apioideae, Apieae) (hereafter *Naufraga*) is a perennial herb (Fig. 1A) inhabiting shady, humid sites on calcareous coastal cliffs, 25–250 m above sea level (Fridlender, 2001; Bibiloni and Soler, 2002). Its distribution range encompasses a short (c. 15 km) stretch of the northern Majorcan coast (Fig. 2A). It is a xenogamous, ant-pollinated species (Cursach and Rita, 2012). Flowering starts in April and ends in August, and fruiting occurs from June to September (Rosselló, 2010). Barochory seems to be its only mode of seed dispersal (Fridlender, 2001; Moragues, 2005). Vegetative reproduction by stolons is frequent (Moragues, 2005). A diploid chromosome number of $2n = 20$, with 0–2 accessory chromosomes, has been reported (Castro and Rossello, 2005). Demographic analyses have shown that populations are declining (Cursach et al., 2013; Cursach and Rita, 2013) and that seedling survival is low (Cursach and Rita, 2012). Major threats include interspecific competition, changing climatic conditions, soil erosion, umbel predation, collection and fires (Rosselló, 2010; Cursach and Rita, 2012; Cursach et al., 2013; Cursach and Rita, 2013). A certain degree of herbivore pressure is thought to benefit *Naufraga* populations by reducing interspecific competition (Cursach et al., 2013).

Phylogenetic dating

Spalik et al. (2010) and Banasiak et al. (2013) obtained the first estimates for the divergence time of *Naufraga* based on broad-scale analyses of ITS sequences of subfamily Apioideae. In order to obtain a more precise estimate, a deeper sampling of closely related genera and additional DNA markers are required. To this end, we conducted a dating analysis using sequence matrices from a separate phylogenetic study of the tribe Apieae (Jiménez-Mejías and Vargas, under review). Forty-four nuclear ITS sequences and 44