Evolutionary change in the germination niche between related species within *Neoporteria* clade (Cactaceae) is idiosyncratic to habitat type

El cambio evolutivo en el nicho de germinación de dos especies relacionadas dentro del clado *Neoporteria* (Cactaceae) es idiosincrático al tipo de hábitat

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ABSTRACT

An appropriate germination response to environmental conditions is the first requirement for the establishment of a plant in a determined geographic area; therefore changes in the germination niche may be critical in configuring the current pattern of species distributions. Furthermore, the spread of many Neotropical groups to higher latitudes are constrained by the combination of cold and humid conditions. This raises the question whether evolutionary trends in germination niche are essential for the mechanism that allows the colonization of edge habitats characterized by humid and cold conditions. Here, we evaluated the variation of seed germination probabilities of two species of cacti (*Neoporteria* clade), closely related but endemic to habitats with different temperature and water availability conditions. The germination niche and differences in germination performance of *Eriosyce subgibbosa* and *E. villosa* were evaluated, using common garden experiments and varying temperature and soil water potential (Ψ) conditions. Seeds of *E. subgibbosa* showed overall higher germination probabilities compared to *E. villosa*. Interestingly, most of the detected differences on seed germination performance are related with a higher ability of *E. subgibbosa* seeds to germinate under conditions with more water availability and under colder conditions compared to *E. villosa* seed germination. This study highlights one of the critical features that could be involved in the mechanism that leads to the expansion of the *Neoporteria* clade to more mesic habitats. Particularly the adaptation of the germination niche of *E. subgibbosa* may allow an expansion from an arid habitat that characterized basal species of the group (plesiomorphic state) to more humid and cold habitats.

Keywords: Endemism, ontogenetic niche, biogeography, Mediterranean biome, Chile.

RESUMEN

Una respuesta apropiada a las condiciones ambientales es el primer requisito para el establecimiento de una planta en un área geográfica, por lo tanto cambios en el nicho de germinación podrían ser críticos en configurar el actual patrón de distribución de especies. Además, la propagación de muchos grupos de origen neotropical hacia altas latitudes está limitada por la combinación de condiciones frías y alta humedad. Esto lleva a preguntarse si las tendencias evolutivas en el nicho de la germinación son esenciales en el mecanismo que permite la colonización del borde del hábitat caracterizado por ser frío y húmedo. Por ello, evaluamos la variación en las probabilidades de germinación de dos especies de cactáceas (clado *Neoporteria*), cercanamente relacionadas pero endémicas a hábitats con diferentes condiciones de temperatura y disponibilidad de agua. Usando experimentos de jardín común se varió la temperatura y el potencial osmótico del sustrato (Ψ), se caracterizó el nicho evaluando las diferencias germinativas en *Eriosyce subgibbosa* y *E. villosa*. Las semillas de *E. subgibbosa* mostraron en general mayor germinación que las semillas de *E. villosa*. Interesantemente, la mayoría de las diferencias detectadas en la germinación de semillas están relacionadas con la mayor habilidad de las semillas de *E. subgibbosa* a germinar bajo condiciones de mayor disponibilidad hídrica y menores temperaturas comparado con la germinación de *E. villosa*. Este estudio llama la atención sobre uno de los aspectos críticos que podrían estar involucrados con el mecanismo que podría llevar la expansión del clado *Neoporteria* a hábitats más mésicos. Particularmente la adaptación del nicho de germinación de *E. subgibbosa* podría permitir la expansión en la distribución de un habitat árido que caracteriza a las especies basales del grupo como *E. villosa* (carácter plesiomórfico) hacia hábitats más húmedos y fríos.

Palabras clave: Endemismo, nicho ontogenético, biogeografía, bioma Mediterráneo, Chile.

INTRODUCTION

The ecological niche is a pivotal concept in ecology, evolution and biogeography, allowing to understand the processes related to survival, reproduction and dispersal of species (Holt 2009, Soberón & Nakamura 2009, Guerrero *et al.* 2013). Part of the importance of this concept relies on the ability of organisms to conserve (or not) their ecological niches (Wiens *et al.* 2010). Within the various factors that define the species niche, those related with early stages of life cycles largely determine the population dynamics of species (Grubb 1977, Poorter 2007). These stages are also critical because they determine the fitness of individuals, and thus are target of natural selection allowing adaptive changes (Post 2003). For example, changes associated with requirements for seed germination are regarded as important evolutionary forces for species living under different climatic conditions (Cruden 1974).

The germination of seeds is one of the earliest ecological processes that determine the occurrence of a species in a plant community. To recruit a new individual into a population, a set of specific environmental conditions is required for seed germination in the place where seeds are dispersed (Donohue *et al.* 2010, Braz *et al.* 2014). Seed germination is a process that depends on the microenvironment experimented by seeds (Baskin & Baskin 2014). In this sense, environmental variables that configure the micro-habitat where occurs seed germination is known as the ´germination niche´ (Harper *et al.* 1965, Donohue *et al.* 2005). Species with a broader germination niche may germinate in a wider range of environmental conditions than species with a narrow niche breadth (Luna & Moreno 2010, Braz *et al.* 2014). An appropriate seed germination response to the microenvironment experimented by seeds is the first requirement for the establishment of a plant in a particular habitat (Donohue *et al.* 2005). Therefore, changes in germination niche among species of a clade may be an adaptive response to different environmental conditions (Cruden 1974).

Despite the strong relationship between germination niche and the ecological divergence of species, the role of seed germination in contributing to species ecological breadth and geographic distribution is still a matter of debate (Rodrigues-Marques *et al.* 2014). Also, the evolutionary trends of germination niche among relatives are far from been understood. Only recently has there been an interest in addressing the conservatism of regeneration niche traits related to seed germination (Walck & Hidayati 2004, Burns & Strauss 2011). For instance, when there is a niche change between relatives it is considered labile, whereas when this feature does not change between related species is considered that the niche is conserved (Losos *et al.* 2003). In general, neotropical lineages which have marked niche conservatism cannot colonize cold and rainy zones (Wiens & Donoghue 2004). For example, the cactus lineage (family level) is distributed from Canada to Patagonia, covering a large set of climates (Anderson 2001), but it is absent in the wettest and coldest areas of the continent. It seems that the combination of low temperatures and excessive rainfall, severely constraints the distribution of cacti, thus avoiding the colonization of southwestern South America (Guerrero *et al.* 2011a). The relationship between germination niche and distribution of cacti is fairly unknown, but there is an important amount of information showing that seed germination of most cacti decreases at low temperature (< 20°C), severe aridity (Guerrero 2011), and absence of light (Rojas-Aréchiga & Vásquez-Yañes 2000, Guerrero *et al.* 2011b).

The genus of cacti *Eriosyce sensu lato* (Kattermann 1994) from Notocacteae tribe, occupies arid and sem-arid biomes. Interestingly, *Eriosyce subgibbosa* (Haw.) Katt. can live in conditions of higher humidity and lower temperatures compared to any other species of Notocacteae. This has led us to characterize and compare germination niche of two species of clade *Neoporteria*, currently within the *Eriosyce sensu lato* group. *Eriosyce villosa* (Monv.) Katt. was choose since is a basal species within the *Neoporteria* clade that occupies arid habitats in the coastal desert of Chile (Guerrero *et al.* 2011c). In addition, we studied the germination niche of *E. subgibbosa* a derived species in relation to *E. villosa*. The objective of this study is to characterize and compare variation pattern of the germination niche between two related *Eriosyce* species of the *Neoporteria* clade, and test whether these differences are in accordance with the environmental characteristics of their habitats.

METHODS

Eriosyce subgibbosa and *E. villosa* belong to the same clade of cacti, but occupy different habitats. *Eriosyce villosa* occupies a fraction of the Desert-Coastal Mediterranean bioclimate (Fig. 1), and *E. subgibbosa* mainly occupies the coastal zone of oceanic-pluviseasonal mediterranean bioclimate (Luebert & Pliscoff 2006). These two bioclimates differ, among other factors, on temperature and rainfall conditions (Fig. 1). These differences account for major abiotic habitat conditions where the germination of the two studied species occur in nature, in contrast, soil properties even that may have some differences they tend to be similar since these species are specialized to regenerate in the crack of coastal rocks (Guerrero unpublished data).

Seeds of these two species were obtained from natural populations in central Chile from mature fruits of fifteen individuals per species, between October and December 2009. In the case of *E. villosa* seeds were collected at the locality of Huasco (28.467°S; 71.219°W), while *E. subgibbosa* seeds were collected at Punta de Lobos in

Pichilemu (34.426°S; 72.043°W). Seeds from both species were pooled and maintained during five (*E. villosa*) and three (*E. subgibbosa*) months in dry conditions until the start of the experiments. To characterize the germination niche a factorial experiment was conducted using two treatments: species (*E. subgibbosa* and *E. villosa*), and two treatments each with several levels of mean daily temperature (6.5°C, 13°C, 18.5°C and 24.5°C) and soil water potential (Ψ: -0.25, -0.5, -1 and -1.5 Mpa). Temperature varied following day/ night cycles as photoperiod (12 / 12 h): 6.5°C (day: 4°C / night: 9°C), 13°C (11° / 16°C), 18.5°C (16° / 21°C) and 24.5° C (22° / 27°C). The criteria to choose thermoperiods in the experimental design were (i) include a wide range of temperature conditions, (ii) use realistic values of maximum and minimum temperature variation that seeds may experience in their habitats (using Huasco and Pichilemu as references, Fig. 1) within a year, and (iii) maintain the same amplitude of variation between the highest and the lowest temperature. The effect of soil water potential was

evaluated using a range of concentrations of mannitol diluted in deionized water (0, 0.1, 0.2, 0.4 y 0.6 mol / L), to generate osmotic potentials in the substrate of 0, -0.25, -0.5, -1 and -1.5 Mpa. Higher concentrations of mannitol generate more negative osmotic pressures and therefore water is less available for seeds (Cárdenas & Villegas 2002). We did not include -1.5 Mpa water potential into statistical analyses because we did no obtain germination at all. Each combination of treatment and level was replicated in five and ten seeds per replicate were sown in petri dishes. We sowed ten seeds in closed petri dishes, on 2 g of vermiculite and deposited in a germination chamber that have fluorescent lights, control temperature with ±0.5°C of precision and with polyurethane temperature isolation. All treatments were watered with deionized water at the start of the experiment and then once a week (maintaining mannitol concentrations of each treatment). The effects of solutes upon water potentials (Ψ) was calculated based on the expression: Ψ= -RTCs, where R is the ideal gas constant,
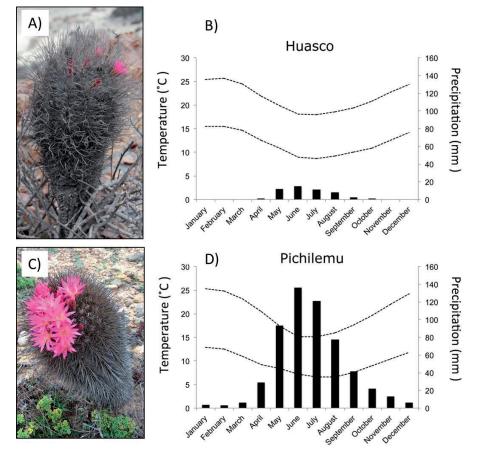

FIGURE 1. Species included in the study and averaged bioclimatic variables (records from the 1950-2000 period) of Huasco and Pichilemu (climatic data from Hijmans *et al.* 2005). A, *Eriosyce villosa*; B, monthly temperature and precipitation at Huasco (28.467°S; 71.219°W); C, *E. subgibbosa*; D, monthly temperature and precipitation at Pichilemu (34.426°S; 72.043°W).

FIGURA 1. Especies incluidas en el estudio y variables bioclimáticas promediadas (registros del periodo 1950-2000) de Huasco y Pichilemu (datos climáticos de Hijmans *et al.* 2005). A, *Eriosyce villosa*; B, temperatura mensual y precipitación en Huasco (28.467°S; 71.219°O); C, *E. subgibbosa*; D, temperatura mensual y precipitación en Pichilemu (34.426°S; 72.043°O).

T the absolute temperature, and Cs the osmolarity of the mannitol solution (Swagel *et al.* 1997). Germination was recorded until no new germinated seeds were observed during three consecutive weeks, taking two months in total. Seeds were considered viable if at the end of the experiment they maintained their hardness and the embryos were white and turgescent. Using this criterion we observed that all seeds were viable.

Significant differences between the germination of both species at different temperatures and soil water potential were tested, performing a three way ANOVA in STATISTICA 7 (StatSoft 2004), where the response variable was the probability of germination and independent variables were temperature, soil water potential and species. The probabilities of germination were transformed to arcsine to meet the normality data assumptions (Zar 1999).

RESULTS

Seed germination of both species varied greatly among the different levels of temperature and osmotic water potential (Figure 2). The maximum germination of seeds was detected at the highest temperatures (16˚ / 21˚C and 22˚ / 27˚C) and osmotic water potentials (0, -0.25, -0.5). In contrast, at osmotic water potentials less than -1, a germination of less than 20% was obtained under all temperatures. Seeds of *E subgibbosa* showed an overall higher germination probabilities than *E. villosa* (F = 12.44, p < 0.0001, Figure 2). Also, seed germination varied significantly between soil water potentials (F = 162.20, p < 0.0001) and among the three temperature conditions (F = 73.67, p< 0.0001). Furthermore, differences were significant for the interaction between species and soil water potential (F = 4.51, p =
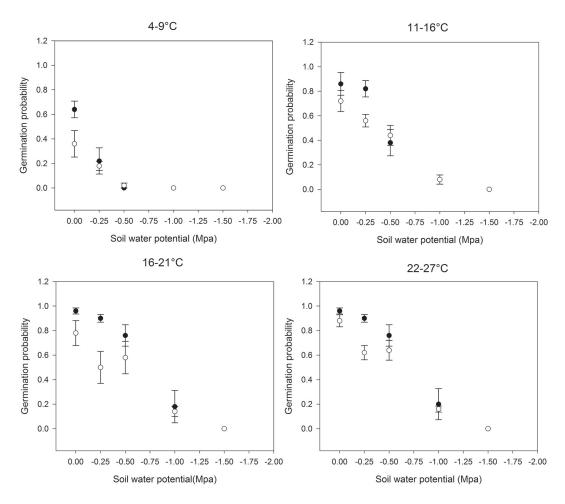

FIGURE 2. Probability of germination of *Eriosyce subgibbosa* (black circles) and *E. villosa* (white circles) under different temperature and soil water potential (mean and S.E.).

FIGURA 2. Probabilidad de germinación de *Eriosyce subgibbosa* (círculos negros) y *E. villosa* (círculos blancos) bajo temperatura y potencial osmótico del suelo diferentes (media y E.S).

0.0013) and among soil water potential and temperature (F = 8.03, p < 0.0001). The interaction between species and temperature (F = 1.74, p = 0.15) and the triple interaction between species, temperature and soil water potential were not significantly different (F = 1.09, p = 0.36).

DISCUSSION

The results indicate that the two studied species of the clade *Neoporteria* (*E. subgibbosa* and *E. villosa*) varied their germination response to different conditions of temperature and soil water potential. In particular, this difference is given because *E. subgibbosa* can germinate more than *E. villosa* especially under higher water availability (higher soil water potential) (Fig. 2). The higher germination of *E. subgibbosa* under conditions of high water availability compared to *E. villosa*, suggests the existence of an adaptive response of *E. subgibbosa* to the idiosyncratic weather conditions where it is distributed. This ability allows *E. subgibbosa* to take advantage of the rainfall in its habitat because precipitation is particularly abundant during cold months (Fig. 1). A fast response to rain may be advantageous because allows seedlings develop early and grow to a size that enables them to withstand the subsequent dry period (Fenner & Thompson 2005). In the germination of both species a decrease in germination was observed in conditions of less soil water potential, suggesting that aridity could be a severe limitation for both species, thus constraining the expansion of species distributions into hyperarid areas of the core of the Atacama Desert. This decay in seed germination under dry conditions could promote fragmentation of the distribution areas of *Eriosyce* species. Since dry phases of pluvial cycles (*sensu* Latorre *et al.* 2006) could diminish the availability of suitable areas (safe sites) for germination and regeneration (Guerrero *et al.* 2011c). Similar patterns of seed germination responses to soil water potential have been shown in other desert plants as in shrubs (i.e. *Cercidium praecox* (Ruiz & Pav.) Harms and *Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M.C. Johnst. and columnar succulents (*Neobuxbaumia tetetzo* (F.A.C.Weber ex K.Schum.) Backeb. and *Pachycereus hollianus* (F.A.C.Weber) Buxb.) (Flores & Briones 2001).

No significant effect of the triple interaction between species, temperature and soil water potential was detected, this suggest differential responses of species seeds to factors that may be annulling their effects. For example, seed germination of species was similar in relation to temperature, since both species germinated more at higher temperatures. However, a closer look of data revealed that > 50% of seeds of *E. subgibbosa* were able to germinate at low temperature (day: 4°C / night: 9°C),) and high water availability. In contrast to the seeds of *E. villosa* which showed very low germination (<50%) at low temperatures (i.e. day: 4°C / night: 9°C) at any water potential. Similar effects have been reported for cacti in Brazil (Zamith *et al.* 2013). This result suggests that suitable conditions (presence of rainfall) for germination of *E. subgibbosa* seeds are achieved almost throughout the year (Fig. 1). In contrast, seeds of *E. villosa* could germinate only during the autumn-winter months, since historically significant levels of rainfall are recorded only in four months during a year (Figure 1). Consequently, the results of germination niche are consistent with the climatic conditions where these species are distributed. These trends in germination niche suggest an evolutionary history of the *Neoporteria* clade linked to climatic gradient of central Chile (Guerrero *et al.* 2011c).

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