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Research article

Infraspecific classification reflects genetic differentiation in the widespread *Petunia axillaris* complex: A comparison among morphological, ecological, and genetic patterns of geographic variation

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ABSTRACT

Plants are subjected to natural selection during expansion of their geographic range or when facing changes in environmental conditions, which in turn may affect phenotypic diversity. Studies on geographic phenotypic variation provide insight into the evolutionary processes and have long contributed to better understanding of diversification within and among species. *Petunia axillaris* is widely distributed in temperate South America, occurring throughout the Pampas region. The current taxonomy of this species recognizes three allopatric subspecies, which occupy nearly adjacent territories, according to floral morphology: *P. axillaris* subsp. *axillaris*, *P. axillaris* subsp. *parodii*, and *P. axillaris* subsp. *subandina*. In this study, we sampled the three *P. axillaris* subspecies and used both molecular markers (from the plastid and nuclear genomes) and morphological measurements to investigate how genetic diversity and morphological variation correlate to ecological variables and the geographic context. We used different forms of Mantel tests (partial and correlograms) to investigate the geographic distribution patterns in distinct types of similarity/dissimilarity among the populations and their relationships. We also modeled the morphological variation in *P. axillaris* as a function of the genetic marker frequencies in the populations. We found that the morphological differences leading to the recognition of different subspecies of *P. axillaris* reflect historical processes of isolation and that adaptation to different ecological conditions faced by each lineage is perhaps not merely a consequence of phenotypic plasticity. These findings suggest that differences between subspecies could represent an incipient speciation stage.

Introduction

Studies on phenotypic diversity along a geographic distribution have contributed to a better understanding of the diversification process within and among species (Thompson and Cunningham, 2002; Nattero et al., 2011). Geographic patterns of phenotypic variation may reflect several processes, including neutral and adaptive variation (Anderson and Johnson, 2008; Toju, 2008; Pérez-Barrales et al., 2009). Differentiation between populations of a species may result from, among other factors, divergent natural selection (Herrera et al., 2006) or may emerge from a balance between neutral variation and limited dispersal, which is akin to isolation-by-distance (Gould and Johnston, 1972). Moreover, ecogeographic isolation has been suggested to be the pre-eminent mechanism driving speciation (Stebbins, 1970; Nattero et al., 2011). However, in most cases, speciation (i.e., the completion of genetic isolation) may be a long process that involves the emergence of multiple isolation barriers and several microevolutionary processes at the population level.

Species belonging to the *Petunia* Juss. genus (Solanaceae) grow in the temperate and subtropical regions of South America, including Argentina, Uruguay, Paraguay, Bolivia, and Brazil (Stehmann et al., 2009). The genus diverged recently and the species show little neutral genetic differentiation in spite of morphological differences between some of them (Lorenz-Lemke et al., 2010; Segatto et al., 2014). A phylogenetic analysis based on plastid genetic markers divided the species in two clades associated with altitude: lowland

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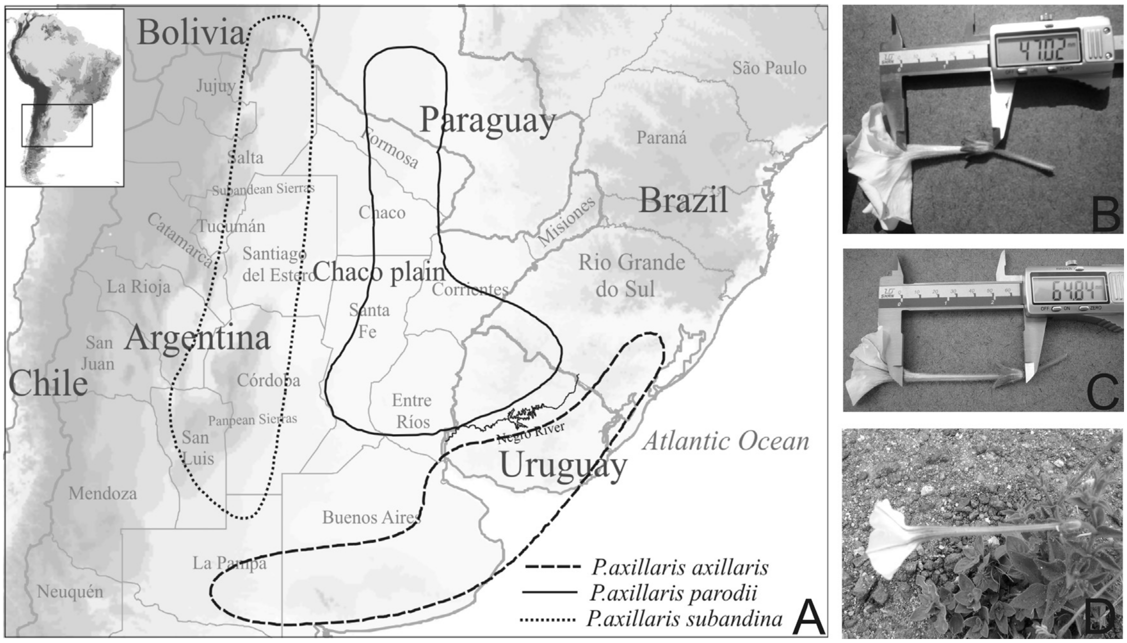

Fig. 1. Subspecies geographic distribution map of *Petunia axillaris* (A) and floral morphology representative of subspecies *axillaris* (B), subspecies *parodii* (C), and subspecies *subandina* (D).

(below 500 m) and highland (above 500 m) groups (Kulcheski et al., 2006; Lorenz-Lemke et al., 2010). The speciation in the highland was influenced by the Pleistocene glacial–interglacial cycles, during which allopatric speciation has been observed (Lorenz-Lemke et al., 2010). The species from the Pampas region are placed in the lowland clade. The glacial–interglacial cycles of the Quaternary and the effects of climatic changes affected differently the lowlands of southern Brazil, where grasslands are dominant (Behling, 2002; Overbeck et al., 2007). Differences in climate, ecology and soil conditions seem to have played an important role in the speciation of lowland *Petunia* clade (Fregonezi et al., 2013).

Although some *Petunia* species are narrowly endemic and associated with specific phytoecological regions, others, such as the *Petunia axillaris* (Lam.) Britton, Sterns and Poggenb complex, are widely distributed in temperate South America (Fig. 1). This complex occurs throughout the entire Pampas region, a geomorphologically complex region that includes several units generated within different tectonic and paleogeographic contexts (Overbeck et al., 2007; Fregonezi et al., 2013). The Pampas is a vast and warm grassland area, covering approximately 500,000 km^2 between the latitudes 29° S and 39° S and including part of the east-central Argentina, the entire territory of Uruguay, and the southern half of Rio Grande do Sul Brazilian state (Pallarés et al., 2005; Fregonezi et al., 2013). Several different physiographic formations composed this region (Bredenkamp et al., 2002), with seasonal forests invading grasslands in the north, especially along streams and river valleys. Psammophytic and halophytic steppes occupy coastal areas, continental dunes and areas with sandy soils, whereas shrubby woodlands generally occur on soils with calcareous crusts and consist of shrubland and thornscrub communities (Quattrocchio et al., 2008).

The *Petunia axillaris* has white flowers, with a corolla that varies between 3 and 7 cm long (Fig. 1) (Stehmann et al., 2009). Hawkmoths appear to be the main pollinators (Ando et al., 1995; Venail et al., 2010; Klahre et al., 2011), although other species have commonly been found visiting the flowers as well (Kokubun et al., 1997; Ando et al., 2001). This species was originally described based on a specimen collected in Montevideo (Uruguay) as part of the *Nicotiana* L. genus and posteriorly included in *Petunia* as *P. nyctaginiflora* (later synonymized to *P. axillaris*). The current taxonomy for this taxon recognizes three allopatric subspecies, *P. axillaris* subsp. *axillaris*, *P. axillaris* subsp. *parodii* (proposed by Cabrera, 1977), and *P. axillaris* subsp. *subandina* (proposed by Ando, 1996). The subspecies occupy nearly adjacent territories (Ando, 1996) and are morphologically distinguishable from each other based on the size of the corolla-limb, length of the corolla-tube, and stamen condition (Fig. 1): subspecies *axillaris* presents flowers with a large limb, short tube, and two long + two medium + one short stamens; subspecies *parodii* has flowers with a small limb, long tube, and four long + one short stamens; subspecies *subandina* flowers have a small limb, long tube, and two long + two medium + one short stamens (Ando, 1996; Kokubun et al., 2006). The morphology of the vegetative organs is similar between the subspecies, though subsp. *subandina* is somewhat taller and more upright than the other two subspecies (Ando, 1996; Kokubun et al., 2006).

Turchetto et al. (2014) analyzed the flower morphology, climatic variables along the geographic distributions, and molecular data from neutral plastid intergenic spacers (cpDNA sequences) and nuclear regions [cleaved amplified polymorphic sequence (CAPS) markers] of the *P. axillaris* complex from wild populations, covering the entire geographic range. The authors found that the *P. axillaris* complex exhibits a wide morphological variation and that the major geographic discontinuities in flower morphology are consistent with the traditional classification of *P. axillaris* subspecies, which in turn is also consistent with niche (climate) divergence. However, although the analyses of the nuclear genome indicated genetic differentiation between the subspecies, the plastid data revealed a low genetic differentiation. This lack of cpDNA differentiation between the subspecies was explained by lineage sorting of ancestral polymorphism due to recent divergence.

The CAPS markers developed for mapping interspecific crosses in *Petunia* are associated with quantitative trait loci (QTLs) for floral morphological traits (Venail et al., 2010; Bossolini et al., 2011; Klahre et al., 2011; Hermann et al., 2013). The six CAPS markers analyzed by Turchetto et al. (2014) are related to morphological characteristics and are located on different chromosomes.