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The long and the short of gene flow and reproductive isolation: Inter-Simple Sequence Repeat (ISSR) markers support the recognition of two floral forms in *Pelargonium reniforme* (Geraniaceae)

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ABSTRACT

The current taxonomy of *Pelargonium reniforme* recognises two subspecies on the basis of habit and vegetative characters, but excludes floral characters. However, populations of *P. reniforme* in the wild tend to belong to easily discernable floral groups based on floral colour and hypanthium length. The aim of this study was to determine the genetic diversity within and between populations of both subspecies using the Inter-Simple Sequence Repeat (ISSR) method of DNA fingerprinting. Ninety individuals from eight populations were sampled. Both phenetic and Neighbor-Net analyses reveal that populations are genetically discernable, but that there is no genetic evidence to support the recognition of the two currently defined subspecies of *P. reniforme*. Instead, the analyses resolved all individuals that possess long hypanthia into a single group, and the role of different pollinators in driving reproductive isolation of this long-tubed form is suggested.

1. Introduction

The genus *Pelargonium* comprises approximately 280 species (Bakker et al., 2004), and 80% of this diversity is centred in the winter rainfall region of the Cape Floral Region of South Africa (CFR; Linder, 2003, 2005). A variety of possible mechanisms for the floristic diversity in the CFR has been proposed (summarised by Linder, 2003), including geographical isolation, phenology, sterility barriers, edaphic factors, climatic specialisation, adaptation to fire and ethology (the role of pollinators). Studies such as those by Johnson (1995, 1997), Johnson and Linder (1995) and Johnson et al. (1998) have emphasised the role that pollinators may play in speciation of the speciose CFR groups. *Pelargonium* has a wide range of floral morphologies (Bakker et al., 2004) but there are only a few studies examining the pollination of members of this genus (McDonald and van der Walt, 1992; Zietsman, 1993; Johnson and Steiner, 1997; Goldblatt & Manning, 2000).

The sectional and species-level phylogenetic relationships of *Pelargonium* have been largely clarified by Bakker et al. (2004) using DNA sequence data. The CFR species form a large, monophyletic clade that is sister to what Bakker et al. (2004) term the "*Peristera*" clade, a group of species from sections *Peristera* and *Reniformia* that extends beyond the CFR and includes non-African species. This clade is also karyologically differentiated from CFR clade, with species possessing x = 11, 19 and 8 as opposed to the CFR clade with x = 10 or 11.

Pelargonium reniforme Curt., which is found in the eastern and central parts of South Africa (Fig. 1), is a member of the *Peristera* clade and has x = 8. While its distribution range overlaps with the CFR, it is not considered a CFR species, and thus

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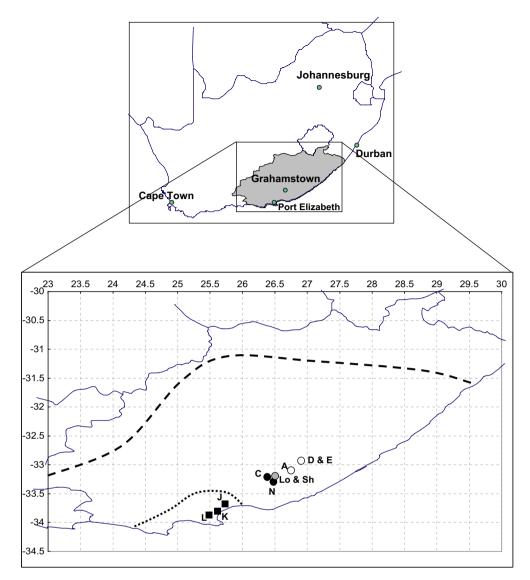


Fig. 1. Map showing the distribution range of *P. reniforme* which is a near endemic to Eastern Cape Province (shaded grey) of South Africa. *P. reniforme* subsp. *velutinum* (- -) is widespread in the eastern cape, while *P. reniforme* subsp. *reniforme* (....) is restricted to the coastal areas around Port Elizabeth. The locations of the eight different populations sampled for the ISSR study are shown and correspond to the labels in Table 1. Closed symbols indicate populations with short hypanthia, open symbols indicates those with long hypanthia and the grey symbol indicates the mixed population. Squares indicate *P. reniforme* subsp. *reniforme*

may be subject to a different suite of selection pressures when compared to CFR taxa. This species is also known to be morphologically variable across its distribution range (Dreyer et al., 1995), suggesting that fixation of traits or adaptations to local conditions may be occurring. Dreyer et al. (1995) recognised four phenotypically distinct groups within the species based on variation of internode length, petiole length, habit and lamina shape. However, only one of these four groups was considered geographically isolated and was recognised as a separate subspecies. *P. reniforme* subsp. *reniforme* (elongated internodes short petioles and reniform leaves) is restricted to the coastal flats near Port Elizabeth (Fig. 1), and *P. reniforme* subsp. *velutinum* (short internodes, longer petioles and leaves seldom reniform) is more widely distributed (Fig. 1). It is worth noting, however, that Dreyer et al. (1995) did not assess these morphological distinctions using morphometric methods. Our phenetic analysis failed to find clear boundaries between specimens assigned to the two putative subspecies. (De Wet, 2007).

The taxonomy of Dreyer et al. (1995) also neglects one of the potentially most important characters of the group: the floral variation. There is an overall lack of distinctive morphological groups in the current taxonomy and an addition of the putatively important floral characters might resolve the taxonomy of the group. Based on herbarium and field observations, we have observed that populations of *P. reniforme* consist of one of two forms: plants with flowers that are either dark pink and long-tubed or pale pink and short-tubed (De Wet, 2007). While our understanding of the pollination biology of these two

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