Genetic variation in invasive populations of sea rocket (*Cakile maritima*) in southern coastal habitats of Australia

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Summary The two congeneric sea rockets (*Cakile maritima* and *Cakile edentula*) have invaded coastal sand dunes in Australia. *C. edentula* was the first species to arrive; however, it almost disappeared and was replaced by the later introduced *C. maritima*. A higher level of variation (genetic and phenotypic) in *C. maritima* is hypothesised to be conducive to its better establishment. To determine the extent and pattern of variation within Australian *C. maritima*, 18 geographic populations were collected and assessed for variation at eight microsatellite loci. The mean allele number per locus was high (7.5), suggesting a high overall diversity across Australia. However, variation was higher within (74%) than among (26%) populations, suggesting genetic differentiation based on geography. Although the species is distributed all along the Australian coast from west to east and north up into Queensland, little evidence of allele flow was detected. In particular, Western Australian populations were distinct from those collected in eastern Australian locations. This indicates several relatively recent and separate introductions of *C. maritima* into Australia.

Keywords Genetic diversity, invasion, microsatellite, multiple introduction.

INTRODUCTION

Australia is an example of an isolated area affected by the invasion of many exotic species. The introduction of some species into Australia has been deliberate (Williams and West 2000); however, sea rocket (*Cakile* species) was introduced accidentally, perhaps through ship ballast. *Cakile maritima* and *Cakile edentula* are members of the Brassicaceae that were introduced to coastal sand dune habitats in Australia, North America, some parts of South America, North Africa, Japan and New Zealand during the late 19th century. Although their invasion causes direct interference with relatively few native plant species, their ecological impacts may facilitate invasion by other species. According to historical records, *C. edentula* was the first of the two species introduced to Victoria, around the 1860s. It spread around the coast of Victoria and South Australia, but was not confirmed further west than Eucla (Western Australia) (Rodman 1986); it has also reached as far north as Mackay (Queensland), and the southern extremities of Tasmania. The first record of *C. maritima* was in the 1890s near Perth (Rodman 1986). This species spread rapidly and colonised the same habitats occupied by *C. edentula*. Nowadays, only *C. maritima* is found throughout the Australian mainland coast and islands: *C. edentula* is restricted to northern New South Wales, Queensland and Tasmania.

Genetic variation leading to adaptation is a key component of successful invasions in many species (e.g. Hardesty *et al*. 2012). Given the out-breeding mating system of *C. maritima* compared to the inbreeding system of *C. edentula*, it is therefore more likely that *C. maritima* will attain higher genetic variation, with consequent adaptive advantage, over *C. edentula*. In addition, the high level of morphological variation in Australian *C. maritima* populations (Cody and Cody 2004; personal observations), has led to a suggestion that high genetic variation may be a result of either introgression by *C. edentula* (as the donor of alleles) into *C. maritima* (as the recipient of alleles) (Cody and Cody 2004) or multiple introductions of *C. maritima* from its European native range. The aim of this study was therefore to evaluate the patterns of genetic variation in Australian *C. maritima* and to explore how the genetic variation may have contributed to its successful invasion.

MATERIALS AND METHODS

Samples were collected from nineteen populations (N = 379 individuals) of *C. maritima* across the Australian mainland from Western Australia (WA, five populations), South Australia (SA, five populations), Victoria (VIC, six populations) and New South Wales (NSW, two populations). For each population, leaves of 20 individuals (at least 20 m apart) were collected and dried at room temperature. Total genomic DNA was extracted from dried leaves using CTAB (Doyle and Doyle, 1987) and the DNeasy Plant Mini Kit (Qiagen, USA).

From a set of microsatellite markers developed for *C. edentula* and *C. lanceolata* by Allan Strand (College of Charleston, North Carolina), 25 loci were chosen and screened for transferability and polymorphism among *C. maritima*. Eight of these were found suitable for our aims and utilised for genotyping all

samples using the primer fluorescent labeling method (Schuelke 2000). PCR products were separated by fragment analysis and then visualised with the use of a 96-capillary ABI 3730 DNA Analyzer at the Australian Genome Research Facility (AGRF). Allele size was analysed using the GeneMapper v4.0 software (Applied Biosystems). The number of alleles, pair-wise F_{ST}, observed heterozygosity, expected heterozygosity and Mantel test were performed using GenAlEx (ver. 6.4, Australian National University). Also pair-wise and population genetic distances were used in a Principal Component Analysis (PCA) to examine the genetic clustering of populations respectively by using GenAlEx6.4.

RESULTS

The eight microsatellite loci amplified a total of 62 alleles (average 7.75) across all populations. The number of alleles per locus ranged from 3 to 12 within populations. This high average number of alleles suggests that *C. maritima* populations are relatively diverse. However, all loci showed some level of heterozygote deficit for all populations (Average F_{IS} 0.49) which was not expected for an out-crossing species. This might be the result of the linear structure of populations and local population isolation in coastal habitats which leads to crossing only between a few close neighbours.

The lowest observed heterozygosity was seen in Geraldon, WA (9.26%) and the highest in Port Fairy, VIC (43.48%). Overall, the amount of observed heterozygosity increased from west (18% for WA) to east (33% for NSW). In addition, pairwise F_{ST} showed significant differentiation between populations, especially between WA populations and the eastern populations (SA, VIC and NSW). According to ANOVA, 74% of the variance was contributed to within population variation, whereas just 26% occurred among populations.

Comparison of geographical and genetic distances between populations showed a significant correlation based on the Mantel test. Principal component analysis (PCA) separated the clusters of SA, VIC and NSW populations from those of WA (the two first axes explained 54.55% and 15.93% of the genetic variation among populations, for the total of 70.74%); however, a single population from Geraldton separated out as an individual population.

DISCUSSION

In general, a strong geographical structure was observed. Although historical records suggest that *C. maritima* was introduced to WA and from there spread to SA, VIC and more eastern parts, the higher genetic variation observed in the eastern populations suggests that diversity in the east cannot be sourced only from WA populations. A strong possibility is that the higher variation has resulted from multiple introductions from different parts of its native range. However, we cannot discount the possibility that variation was introduced by hybridisation between *C. maritima* and *C. edentula* where they have coexisted for short periods in eastern Australia. Putative hybrids have been reported. Further study is required to distinguish between these alternatives.

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