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INVASION NOTE

Cytogeography of *Oxalis pes-caprae* in its native range: where are the pentaploids?

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Abstract Due to its instantaneous effects on the genetics, phenotype, physiology and/or ecology of a plant, polyploidy can play an important role in facilitating plant invasions. Understanding the determinants of invasiveness in species with multiple ploidy levels requires a detailed knowledge of ploidy composition in native versus invaded ranges. Using DNA flow cytometry, we performed representative ploidy screening (277 localities, 333 individuals) across the native range of *Oxalis pes-caprae* and compared the data with those from invaded ranges. Both ranges showed striking differences in ploidy composition: whereas tetra- and especially pentaploids successfully colonized secondary areas, only di-, (very rare) tri- and tetraploids (dominant) were found in the native range of this species. Disregarding the diploid var. *sericea*, diploids and tetraploids of the nominate variety showed largely parapatric distribution in the native range, with a zone of overlap in the Northern Cape Province. Our results challenge the conventional scenario of the introduction of pentaploid individuals from the Greater Cape Floristic Region. The origin of the pentaploid cytotype is unclear and molecular tools applied in a large scale screening are needed to understand the invasion history of the species.

Keywords Cytogeography · Flow cytometry · Greater Cape Floristic Region · *Oxalis pes-caprae* · Polyploidy

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Introduction

Polyploidy (i.e., the presence of more than two complete chromosomal sets in a somatic cell) can play an important role in facilitating plant invasions (Pandit et al. 2011). The success of polyploid plants can be related to genetic, phenotypic, physiological and/or ecological characteristics that may alter their vigour, contribute to local adaptation after introduction and ultimately lead to their spread in novel

environments (te Beest et al. 2012). Specifically, polyploids are often more robust, have higher biomass, produce larger seeds, and show higher phenotypic plasticity. Physiological alterations can result in increased stress tolerance (e.g., drought or cold resistance) and thus broader ecological amplitude (Levin 2002). Biotic interactions, including parasites and/or pathogens, can also be changed due to changes in the quality and quantity of secondary metabolites associated with genome duplication (Hull-Sanders et al. 2009). The genetic superiority of polyploids involves increased heterozygosity, which affords opportunity to mask deleterious alleles and reduce the cost of inbreeding, increased genetic diversity (more alleles per locus), or enhanced gene expression (Ramsey and Schemske 2002). These life history traits may predispose polyploids to colonization success in secondary areas. Indeed, in many species with ploidy heterogeneity in their native range, only polyploids are present in the invaded ranges of such taxa (e.g., Kubátová et al. 2008; Schlaepfer et al. 2010). Genome duplication after introduction into novel environments, which may result in the evolution of invasiveness, has also been reported, although less frequently (e.g., Mandák et al. 2003). Better understanding of the determinants of invasiveness in species with multiple ploidy levels requires a detailed knowledge of ploidy composition in both the native and invaded ranges.

The Bermuda buttercup (*Oxalis pes-caprae* L., Oxalidaceae) is a ploidy-heterogeneous geophyte native to the Greater Cape Floristic Region (GCFR) of South Africa (Goldblatt and Manning 2000; Born et al. 2007), with the centre of distribution in the Western and Northern Cape Provinces (Salter 1944). The species has become one of the most invasive plants in several Mediterranean areas of the world, and also extends to subtropical and semiarid regions. *Oxalis pes-caprae* has been reported as a noxious weed from southern Europe, North Africa, SW Asia, China, Japan, Australia, New Zealand, several states of the USA (Arizona, California and Florida), and western South America (Lambdon 2006). Two morphologically distinct varieties (var. *pes-caprae* and var. *sericea* (L.f.) T.M.Salter) are recognized in the GCFR, but only the nominate variety seems to be introduced outside of its native range. While both sexual (requiring legitimate pollination of tristylous flowers) and vegetative (via bulbil formation) propagation is known from the native range, the latter clearly prevails throughout the invaded ranges of this species. Vegetative propagation is often the only mode of reproduction due to the absence of cross-compatible floral morphs. Investigations into ploidy variation of *O. pes-caprae* (Ornduff 1987; Castro et al. 2007) revealed that a short-styled pentaploid morph dominated in its exotic range, but was apparently rare in the GCFR (Ornduff 1987). In fact, there is only one record of pentaploid plants from the native range (Franklin in Michael 1964). Unfortunately the current knowledge of ploidy variation in the native range of this species is still based on very limited sampling.

To obtain a holistic view of ploidy variation across the native range of *O. pes-caprae* and to identify putative source populations of pentaploids that would allow comparative experimental study between native and invasive plants with the same level of ploidy, we used DNA flow cytometry to estimate ploidy levels in a representative set of samples.

Methods

Sampling

A total of 333 plants of *O. pes-caprae* from 277 localities in the Western and Northern Cape provinces of the GCFR (spanning 29°13′S–34°50′S and 17°25′E–23°09′E; Fig. 1, see Supplemental Table 1 for locality details) were screened for ploidy variation. Although only the nominate variety was likely introduced outside of the GCFR, we cytotyped both varieties (var. *sericea*: 41 individuals, 27 localities; var. *pes-caprae*: 292 individuals, 266 localities) because (1) they were not always distinguished previously, (2) hybridization between both varieties has been suspected (Salter 1944), and (3) there were no previous ploidy data for var. *sericea*. The samples were collected during 2007–2012. Either fresh leaf material from plants grown from bulbs at the Experimental Garden of the Institute of Botany, Academy of Sciences of the Czech Republic in Průhonice (85 samples), or dehydrated leaf tissue that was silica-dried in the field (248 samples), were used for flow cytometric analyses.

Flow cytometry

Relative fluorescence intensities of isolated nuclei were determined using 4′,6-diamidino-2-phenylindole

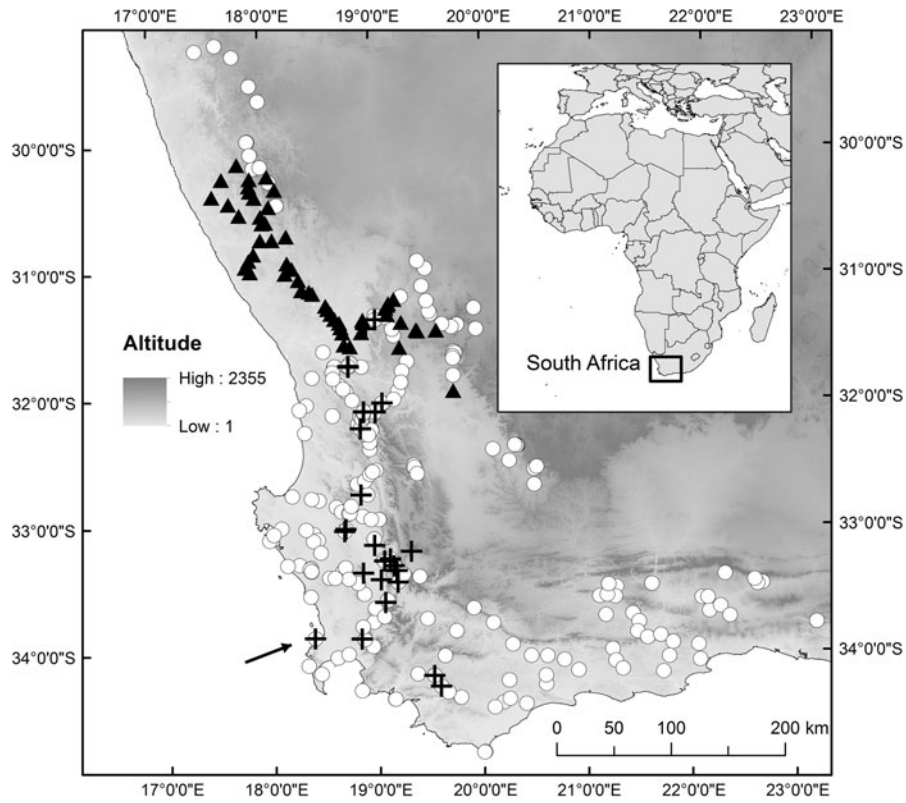

Fig. 1 Ploidy distribution of *Oxalis pes-caprae* in Western and Northern Cape provinces. *Closed triangle* diploid var. *pes-caprae*; *open circle* tetraploid var. *pes-caprae*; *cross* diploid var. *sericea*. One triploid population of var. *sericea* is indicated by an *arrow*

(DAPI) flow cytometry as described in Doležel et al. (2007). A two-step simplified procedure using Otto buffers was selected and fresh *Glycine max* 'Polanka' (2C = 2.50 pg) served as an appropriate internal reference standard. The recorded values were compared to flow cytometric results of Castro et al. (2007) to infer DNA ploidy levels of the samples. Pentaploid plants from the Iberian Peninsula were also used as a reference. The flow cytometric measurements were analyzed using the GLM procedure in the Statistica ver. 10.

Results

Flow cytometric analyses of fresh and dry *O. pes-caprae* leaf tissue resulted in distinct peaks of relative fluorescence intensities of both sample and internal standard, and low background noise. Mean coefficients of variation of G0/G1 peaks of *Oxalis* samples and reference standards were 4.15 % (fresh samples—3.04 %, dry samples—4.57 %) and 1.97 %, respectively. The sample/standard fluorescence ratios formed three well-separated groups, corresponding to diploid, triploid and tetraploid plants, respectively (Table 1). Triploids of *O. pes-caprae* were detected for the first time. Fresh and desiccated tissues yielded nearly identical fluorescence values (mean ± SD: 0.286 ± 0.009, n = 21 vs. 0.286 ± 0.010, n = 67; $F_{1,86} = 0.000$, $p = 0.995$ and 0.564 ± 0.011, n = 50 vs. 0.562 ± 0.014, n = 181; $F_{1,229} = 2.087$, $p = 0.150$ for diploids and tetraploids, respectively). Diploids of var. *sericea* showed marginally significant differences ($F_{1,86} = 4.226$, $p = 0.046$) in fluorescence intensities as compared to those of var. *pes-caprae* (Table 1).

Most of the samples identified as *O. pes-caprae* var. *sericea* were diploid. They were recorded at 26 localities distributed along the Atlantic coast of the Western Cape Province in the GCFR (Fig. 1). One uniformly triploid population of var. *sericea* was collected on Signal Hill in Cape Town. Interestingly, populations of *O. pes-caprae* var. *sericea* were often found in sympatry with tetraploid populations of var. *pes-caprae* (16 out of 27 localities). The nominate variety was ploidy-heterogeneous and comprised of

Table 1 Flow cytometric results of 333 analyzed samples of *Oxalis pes-caprae* from the native range of distribution

Oxalis pes-caprae	Ploidy level	Relative fluorescence intensity (mean ± SD)	Number of populations/ number of individuals
var. *pes-caprae*	2x	0.285 ± 0.008	54/61
var. *pes-caprae*	4x	0.563 ± 0.013	212/231
var. *sericea*	2x	0.290 ± 0.012	26/27
var. *sericea*	3x	0.447 ± 0.004	1/14

Isolated nuclei were stained with DAPI and *Glycine max* 'Polanka' (2C = 2.50 pg) was set as the unit fluorescence value

diploids (54 localities) and tetraploids (212 localities) that were more-or-less parapatric (Fig. 1). Diploids are centered in the arid Knersvlakte, with extensions to the Bokkeveld Plateau in the east and Namaqualand in the north. The contact zone with tetraploids runs through the Kamiesberg and Bokkeveld mountains. Surprisingly, no plants with fluorescence intensity corresponding to pentaploids were found in the native range of the species.

Discussion

Our study aimed to contribute to the cytogeography of *O. pes-caprae* across its native range. We were particularly interested in the native range and distribution of pentaploids, as this ploidy level has become a noxious weed in several Mediterranean-type regions across the world. Ploidy variation of *O. pes-caprae* in invaded regions has been recently studied (see Castro et al. 2007 and references therein), but knowledge of the cytology of the species in its native range is confined to a handful of chromosomal counts (Marks 1956; Franklin in Michael 1964; Ornduff 1987).

We found striking differences in ploidy composition between native and invaded ranges. Whereas tetra- and pentaploid plants occur in the invaded range and the latter clearly prevails (pentaploid chromosome counts are recorded from several countries of the Mediterranean basin, India, California, South and Western Australia; Ornduff 1987; Castro et al. 2007), the native range is inhabited by di- and tetraploids (in addition to one triploid population of predominantly diploid *O. pes-caprae* var. *sericea*). Diploids and tetraploids of the nominate variety have distinct distribution patterns (parapatry with zones of cytotype contact), as well as slightly different phenologies (diploids usually start flowering before tetraploids; our field observations). There are also ecological differences between the cytotypes, with diploids being restricted to more arid habitats dominated by Succulent Karoo vegetation. Diploid populations of var. *pes-caprae* were never recorded in Fynbos vegetation. On the contrary, tetraploids clearly prevailed in the Fynbos Biome and inhabited sites with Fynbos or Renosterveld vegetation (Mucina and Rutherford 2006). We did not find any pentaploid individuals in South Africa, despite intensive sampling (more than 300 plants were cytotyped in total, with nearly 30 from five localities in Cape Town where the incidence of pentaploid plants was assumed based on publications). This result was quite unexpected, because previous karyological records for approximately a dozen plants from the native range indicated the presence of di-, tetra- and pentaploids (Marks 1956; Franklin in Michael 1964; Ornduff 1987). The only pentaploid among these published was collected in the immediate vicinity of Cape Town (Franklin in Michael 1964). Unfortunately the non-availability of the voucher microscope slide precludes confirmation of this published pentaploid chromosome count. It should be noted that karyological analysis of *Oxalis* is difficult and fragile chromosomes and presence of satellites can lead to an overestimation of the number of chromosomes (H. Schneeweiss et al., in preparation).

The potential for weedy behaviour of the different varieties and cytotypes of *O. pes-caprae* in the GCFR is of interest. The original distribution range of *O. pes-caprae* has been complicated by extensive colonization of anthropogenically-altered habitats, but our field observations shed some light on possible natural range size and spread. Variety *sericea* shows little evidence of weedy behaviour, as it mostly occurs in natural habitat within the Fynbos Biome and usually all three morphs are present in a population. Of the nominate variety, the higher potential for spreading in the natural range is the tetraploid; this often grows in disturbed and man-made habitats, forms large monospecific stands, and often lacks some floral morphs in populations (our field observations). The spreading potential of diploids of var. *pes-caprae* appears to be intermediate, but large populations of this cytotype were seldom encountered. For example, in the

Nieuwoudtville area where both cytotypes come into contact, arable land appears to be inhabited by tetraploid while natural vegetation by diploid plants. Thus, the potential of diploids for weedy behaviour in the native range seems to be lower than that of tetraploids. This could indicate that current distribution ranges of the diploid cytotypes of *O. pes-caprae* are more reliable indicators of the past range of this species.

The apparent absence of pentaploids in the native range, together with the detailed ploidy distribution pattern presented here, raises questions about the place and mechanism of origin of the most invasive cytotype of *O. pes-caprae*. The generally accepted view is that pentaploidy originated in South Africa as a result of fusion of an unreduced gamete of the tetraploid and a reduced gamete of the diploid cytotypes (Ornduff 1987). There are marked morphological differences between the two varieties (var. *pes-caprae*, var. *sericea*), and the morphology of the invasive pentaploids matches the nominate variety. The hypothesis that the pentaploid arose through hybridization between diploid individuals of var. *sericea*, which do often grow in sympatry with tetraploids of var. *pes-caprae*, thus seems unlikely. If the syngamy between di- and tetraploid plants is accepted, the most likely place of pentaploid origin is the Kamiesberg mountains or the Bokkeveld Plateau. Diploid and tetraploid individuals of *O. pes-caprae* var. *pes-caprae* were found growing less than 3 km apart in the Nieuwoudtville area, and mixed-ploidy populations may occur. The Bokkeveld Plateau is often referred to as the "bulb capital of the world". This richness in horticulturally valuable geophytes has led to extensive plant collecting in the area (Manning et al. 2002). The accidental transport of *O. pes-caprae* bulbs in horticultural collections and their further export to different areas of the world thus seems possible. However, our sampling in contact zones did not reveal other ploidy of var. *pes-caprae* except diploid and tetraploid.

Another possible mode of pentaploidy genesis involves hybridization between tetraploid and putative hexaploid plants, the latter originating from unreduced and reduced gametes of the tetraploid. Although such a scenario would extend the possible area of origin of pentaploids to almost the entire GCFR, including the more densely-populated southwestern area (which experiences elevated international transport rates and increased probability of alien plant transport), the lack of hexaploids weakens this hypothesis. The same drawback is associated with the alternative hypothesis that would assume the genesis of pentaploids from the introduced tetraploids in one or more regions of their secondary range. Tetraploid plants are known to occur in several areas of the invaded range, including Portugal, Morocco, India and South and Western Australia (Ornduff 1987; Castro et al. 2007). Populations with tetraploid and pentaploid plants growing in sympatry have also been reported (Castro et al. 2007, 2009).

In summary, a detailed ploidy screening in the native range of *O. pes-caprae* failed to reveal any pentaploids, which prevail across invaded ranges. Comparative studies between native and invasive pentaploids are therefore not possible at this stage of investigation. The putative lack of pentaploids in the GCFR challenges the widely accepted scenario, which places the origin of the invasive cytotype to the native range. The origin of the pentaploid *O. pes-caprae* is currently unknown and molecular tools applied in a large scale screening are needed to understand the invasion history of the species.

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References

Born J, Linder HP, Desmet P (2007) The greater cape floristic region. J Biogeogr 34:147–162

Castro S, Loureiro J, Santos C, Ater M, Ayensa G, Navarro L (2007) Distribution of flower morphs, ploidy level and sexual reproduction of the invasive weed *Oxalis pes-caprae* in the western area of the Mediterranean region. Ann Bot 99:507–517

Castro S, Loureiro J, Sousa AJ, Rodriguez E, Santos C, Ayensa G, Navarro L (2009) Is the heterostylous *Oxalis pes-caprae* able to reproduce sexually in the invasive range? In: Proceedings of the world conference on biological invasions and ecosystem functioning, 27–30 Oct 2009, Porto, Portugal

Doležel J, Greilhuber J, Suda J (2007) Estimation of nuclear DNA content in plants using flow cytometry. Nat Prot 2:2233–2244

Goldblatt P, Manning J (2000) Cape plants. A conspectus of the Cape Flora of South Africa. Strelitzia 9. SANBI, Pretoria, South Africa

Hull-Sanders HM, Johnson RH, Owen HA, Meyer GA (2009) Effects of polyploidy on secondary chemistry, physiology, and performance of native and invasive genotypes of *Solidago gigantea* (Asteraceae). Am J Bot 96:762–770

Kubátová B, Trávníček P, Bastlová D, Čurn V, Jarolímová V, Suda J (2008) DNA ploidy-level variation in native and invasive populations of *Lythrum salicaria* at a large geographical scale. J Biogeogr 35:167–176

Lambdon P (2006) *Oxalis pes-caprae*. DAISIE factsheet (Delivering Alien Invasive Species Inventories for Europe). http://www.europe-aliens.org/pdf/Oxalis_pes-caprae.pdf. Accessed 19 Oct 2012

Levin DA (2002) The role of chromosomal change in plant evolution. Oxford University Press, Oxford

Mandák B, Pyšek P, Lysák M, Suda J, Krahulcová A, Bímová K (2003) Variation in DNA-ploidy levels of *Reynoutria* taxa in the Czech Republic. Ann Bot 92:265–272

Manning J, Goldblatt P, Snijman D (2002) The color encyclopedia of Cape bulbs. Timber Press, Portland

Marks GE (1956) Chromosome numbers in the genus *Oxalis*. New Phytol 55:120–129

Michael P (1964) The identity and origin of varieties of *Oxalis pes-caprae* L. naturalized in Australia. Trans R Soc S Aust 88:167–173

Mucina L, Rutherford MC, eds (2006) The vegetation of South Africa, Lesotho and Swaziland. Strelitzia 19. SANBI, Pretoria, South Africa

Ornduff R (1987) Reproductive systems and chromosome races of *Oxalis pes-caprae* L. and their bearing on the genesis of a noxious weed. Ann Mo Bot Gard 74:79–84

Pandit MK, Pocock MJO, Kunin WE (2011) Ploidy influences rarity and invasiveness in plants. J Ecol 99:1108–1115

Ramsey J, Schemske DW (2002) Neopolyploidy in flowering plants. Annu Rev Ecol Syst 33:589–639

Salter TM (1944) The genus *Oxalis* in South Africa—a taxonomic revision. J S Afr Bot Suppl 1:1–355

Schlaepfer DR, Edwards P, Billeter R (2010) Why only tetraploid *Solidago gigantea* (Asteraceae) became invasive: a common garden comparison of ploidy levels. Oecologia 163:661–673

te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubešová M, Pyšek P (2012) The more the better? The role of polyploidy in facilitating plant invasions. Ann Bot 53:19–45