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The spatio-ecological segregation of different cytotypes of *Oxalis obtusa* (Oxalidaceae) in contact zones

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ABSTRACT

Contact zones of different cytotypes provide an opportunity to address evolutionary mechanisms underlying the origin, establishment and maintenance of karyological diversity at intraspecific level. We explored the fine-scale distribution of ploidy levels of *Oxalis obtusa* in seven mixed-ploidy sites in the Western and Northern Cape Provinces, South Africa, and searched for potential isolating barriers promoting assortative mating. Five different ploidy levels (2x, 3x, 4x, 6x and 8x) were detected among 336 samples collected at 112 microsites. The studied sites were inhabited by two (2 sites), three (4 sites) or even all five (1 site) different cytotypes. Despite their sympatric growth, different ploidies show some spatio-ecological segregation. The greatest differences among microsites hosting different cytotypes were found in precipitation parameters. There is a clear altitudinal gradient in ploidy composition in the most ploidy-variable site, Pakhuis Pass. Our results show that a combination of niche partitioning and clumping of same-ploidy individuals due to vegetative reproduction seems to be efficient reproductive barriers, which limit inter-ploidy gene flow in the zones of ploidy contact and contribute to the long-term maintenance of cytotype mixtures in *O. obtusa*.

1. Introduction

Polyploidy is a major evolutionary process in eukaryotes, particularly in angiosperms, where several ancient and recent whole-genome duplication events have been detected (Coghlan et al., 2005; Soltis et al., 2009). Polyploids often differ from their diploid counterparts in a number of molecular, morphological, physiological and ecological characteristics, including quantitative plant traits, growth and developmental rates, flowering phenology, proportion of sexual to vegetative reproduction, and genetic make-up (Comai, 2005; Hegarty and Hiscock, 2007; Otto, 2007). These modifications often enable the polyploids to occupy different ecological niches or a wider range of habitats, resulting in ecological displacement and spatial segregation between diploids and polyploids (Schönswetter et al., 2007; Sonnleitner et al., 2010; Husband et al., 2013).

Although different cytotypes can be recognized as separate taxa (Suda et al., 2007), the concept of intraspecific ploidy variation has become widely adopted in recent years, particularly in autopolyploids (Husband et al., 2013). The overall frequency of angiosperm species with multiple ploidy races is difficult to estimate, because the vast majority has not been subjected to detailed karyological investigation and only a few chromosomal counts per species are usually available. Based on a broad survey of species, Wood et al. (2009) assumed that 12–13% of angiosperm species and 17% of fern species are ploidy-variable. These frequencies are likely to be underestimated, with intraspecific ploidy heterogeneity likely to increase with more intensive sampling. Indeed, representative samplings made possible with the aid of flow cytometry have resulted in a dramatic increase in the number of species known to be ploidy-heterogeneous, as well as in the number of different cytotypes recognized within a species (Kron et al., 2007). Currently, angiosperm species with up to eight different euploid cytotypes are known (Sonnleitner et al., 2010). The pattern of ploidy distribution in situ reflects, among others, ecological preferences of different cytotypes, their dispersal abilities and the dynamics of genome duplication (Petit et al., 1999; Husband et al., 2013). When polyploids first arise, they by necessity occur in sympatry with their diploid/lower-polyploid progenitors. Subsequent cytotype expansion or retreat can result in parapatric (i.e. a spatial arrangement where the boundary of one cytotype abuts against that of another with little overlap) or even allopatric (i.e. mutually exclusive) distributions. The observed distributional pattern also depends on the scale at which the

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investigation is conducted. Whereas observations made at large scales may, for instance, indicate sympatry, a detailed investigation at microsite scale can reveal ecological segregation of different cytotypes (Kolář et al., 2009). True cytotype mixtures are either of temporary nature, with one ploidy being finally outcompeted (i.e. the minority cytotype exclusion principle; Levin, 1975) or persist long-term when inter-ploidy prezygotic or postzygotic reproductive barriers evolve (Husband and Sabara, 2004). Contact zones can either develop as a 'mosaic zone', with different cytotypes patchily distributed along different ecological environments, or as a monotonic cline caused by density-dependent rates of gene flow forming a 'tension zone' (Petit et al., 1999). In contrast to the localized mosaic zones, tension zones are not maintained by the response to local environmental conditions and are therefore to some extent mobile (Petit et al., 1999). Most contact zones are formed by two cytotypes, although recent years have seen the discovery of much more complex population structures, involving up to five different cytotypes (Trávníček et al., 2011).

Currently, *Oxalis obtusa* Jacq. is the most ploidy variable species known to be native to the Greater Cape Floristic Region (GCFR) of South Africa (Krejčíková et al., 2013). This phenotypically distinct species is common in the Western and Northern Cape Provinces, from which it extends to the Eastern Cape Province and southern Namibia (Dreyer and Makgakga, 2003). *O. obtusa* inhabits sites with different abiotic conditions and large altitudinal range. Krejčíková et al. (2013) found three majority (2x, 4x, 6x) and three minority (3x, 5x, 8x) in situ cytotypes in this species, with cytotype distribution correlating significantly with vegetation type. Whereas the most widespread hexaploids dominated in Fynbos vegetation, tetraploids mostly occurred in Succulent Karoo vegetation. In addition to single-cytotype sites, *ca.* 5% of cytotyped populations were ploidy-mixed and different cytotypes were sometimes recorded in localities less than 3 km apart.

Our previous work (Krejčíková et al., 2013) addressed ploidy distribution of this species at a large spatial scale, covering nearly the entire distribution range of *O. obtusa*. To gain further insight into the ploidy structure and potential ecological sorting of different cytotypes at a local scale, we selected seven sites with mixed-ploidy populations, odd ploidy levels or different cytotypes growing in close proximity. We subjected these sites to a detailed ploidy screening, in order to answer the following questions: (i) What is the pattern of cytotype distribution in the zones of ploidy contact? (ii) How frequent are odd ploidy levels that indicate inter-cytotype mating? and (iii) Does ploidy distribution at the local scale reflect abiotic conditions at these microsites?

2. Material and methods

2.1. Field sampling

The plants were collected at seven sites (=localities), four in the Western Cape Province and three in the Northern Cape Province, respectively (Fig. 1). The sites were selected based on the following criteria: (i) the abundance of *O. obtusa* and (ii) the shared presence of different cytotypes in close proximity (i.e. only a few kilometers apart) or physically intermixed and sympatric, based on our previous results (Krejčíková et al., 2013). A linear transect (along the road) was established at five sites, while sampling across an area was performed at two additional sites (Table 1). All but one (Silwerstroomstrand) linear transects were laid out through mountain passes with steep ecological gradients and a wide range in altitudes (340–740 m). Whenever possible and depending on the site, sampling points (=microsites) were spaced approximately 0.5 km apart. If no *O. obtusa* was found at that point, the nearest population was collected and the next sampling point moved accordingly. Additional sampling was done at microsites harboring individuals with distinct morphologies (e.g. giant/dwarf morphotypes, unique flower color, etc.). The number of microsite samplings per locality varied from 4 to 40, reflecting primarily the size of the area under investigation (see the Supplementary data for microsite details). At each microsite, three individuals were collected in an area of approximately 10 × 10 m. Whenever possible, different stylar morphs (long-, middle- and short-styled) were chosen to avoid the collection of genetically identical plants. Leaf tissue of all samples (336 in total) was silica-dried in the field. In addition, bulbs from 93 populations were collected and grown at the Experimental Garden of the Institute of Botany, Academy of Sciences of the Czech Republic in Průhonice (50°00′ N 14°34′ E). A monograph on South African *Oxalis* (Salter, 1944) was used for species identification. Herbarium vouchers are kept in the herbarium of the Charles University in Prague (PRC); voucher numbers correspond to sample numbers provided in the Supplementary data.

2.2. Flow cytometry

DNA ploidy levels were estimated using flow cytometry according to the methodology detailed in Krejčíková et al. (2013). Leaf tissue of the analyzed samples was chopped together with the internal reference standard (*Glycine max* 'Polanka', 2C = 2.50 pg; Doležel et al., 2007) in a Petri-dish containing 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20; Otto, 1990). The crude suspension was filtered through a 42-μm nylon mesh and incubated for *ca.* 15 min at room temperature. Isolated nuclei were stained with 1 ml of Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12\ H_2O$) supplemented with 4′,6-diamidino-2-phenylindole (DAPI) at a final concentration of 4 μg ml^{-1} and β-mercaptoethanol (2 μl ml^{-1}). After a few minutes, the relative fluorescence intensity of at least 3000 particles was recorded using a Partec PA II flow cytometer (Partec GmbH, Münster, Germany) equipped with a mercury arc lamp as UV light excitation source. Histograms were evaluated using Partec FloMax software, ver. 2.4d. Up to three *Oxalis* plants from the same population were analyzed together; each plant was re-analyzed separately if mixed-ploidy samples were found or if the quality of the histograms was not sufficient (i.e. coefficient of variation of any peak above 5%).

2.3. Data analysis

Vegetation, climatic data and altitudinal data were extracted using Arc GIS 10.1 (Esri, Redlands, CA, USA) from the Vegetation Map of South Africa, Lesotho and Swaziland (Mucina and Rutherford, 2006), the South African Atlas of Climatology and Agrohydrology (Schulze et al., 2008) and the SRTM digital elevation model of South Africa. In total, 41 variables related to soil (11 attributes), precipitation (29 attributes) and temperature (one attribute) were assessed (see the Supplementary data). Climatic parameters with the largest differences among the sites harboring different even ploidies (i.e. 2x, 4x, 6x, 8x) were selected by canonical discriminant analysis using SAS 9.3 (SAS Institute, Cary, NC, USA). Canonical discriminant analysis is a dimension-reduction technique that summarizes the between-classes variation (Klecka, 1980; Lepš and Šmilauer, 2003). It derives a linear combination of the variables (abiotic characteristics in our particular case) that produces the greatest distance among the a-priori defined categories (different cytotypes in our case). To control for possible spatial autocorrelation, we also included geographic co-ordinates as independent variables to discriminant analyses. In addition to statistical tests of the entire dataset, a separate analysis was performed for a transect across the Pakhuis Pass, which was the most intensively sampled and most ploidy-variable site.

3. Results

Five different ploidy levels (2x, 3x, 4x, 6x and 8x) were detected among 336 *O. obtusa* samples collected at 112 microsites from seven localities (Table 2). While two different ploidy levels co-existed at two localities in the Northern Cape Province (Ouberg Pass and the vicinity of Nieuwoudtville), three sympatric cytotypes were encountered at four other localities (Table 2). The most ploidy-complex site was the Pakhuis Pass NE of Clanwilliam, where all five cytotypes co-occurred.