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High ploidy diversity and distinct patterns of cytotype distribution in a widespread species of *Oxalis* in the Greater Cape Floristic Region

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• *Background and Aims* Genome duplication is widely acknowledged as a major force in the evolution of angiosperms, although the incidence of polyploidy in different floras may differ dramatically. The Greater Cape Floristic Region of southern Africa is one of the world's biodiversity hotspots and is considered depauperate in polyploids. To test this assumption, ploidy variation was assessed in a widespread member of the largest geophytic genus in the Cape flora: *Oxalis obtusa*.

• *Methods* DNA flow cytometry complemented by confirmatory chromosome counts was used to determine ploidy levels in 355 populations of *O. obtusa* (1014 individuals) across its entire distribution range. Ecological differentiation among cytotypes was tested by comparing sets of vegetation and climatic variables extracted for each locality.

• *Key Results* Three majority (2*x*, 4*x*, 6*x*) and three minority (3*x*, 5*x*, 8*x*) cytotypes were detected *in situ*, in addition to a heptaploid individual originating from a botanical garden. While single-cytotype populations predominate, 12 mixed-ploidy populations were also found. The overall pattern of ploidy level distribution is quite complex, but some ecological segregation was observed. Hexaploids are the most common cytotype and prevail in the Fynbos biome. In contrast, tetraploids dominate in the Succulent Karoo biome. Precipitation parameters were identified as the most important climatic variables associated with cytotype distribution.

• *Conclusions* Although it would be premature to make generalizations regarding the role of genome duplication in the genesis of hyperdiversity of the Cape flora, the substantial and unexpected ploidy diversity in *Oxalis obtusa* is unparalleled in comparison with any other cytologically known native Cape plant species. The results suggest that ploidy variation in the Greater Cape Floristic Region may be much greater than currently assumed, which, given the documented role of polyploidy in speciation, has direct implications for radiation hypotheses in this biodiversity hotspot.

Key words: Cape Floristic Region, cytogeography, flow cytometry, Fynbos, *Oxalis obtusa*, polyploidy, Succulent Karoo, vegetation.

INTRODUCTION

The south-western tip of the African continent hosts a unique and exceptionally rich flora. It includes the Cape Floristic Region (CFR) and the Succulent Karoo Region (Van Wyk and Smith, 2001), which together form the winter-rainfall region known as the Greater CFR (Born *et al.*, 2006). Both areas are recognized as important centres of plant diversity and endemism (Myers *et al.*, 2000). The CFR is home to about 9000 plant species in an area of approx. 90 000 km^2, and it displays a species-level endemism of 68·8 % (Goldblatt and Manning, 2000). Such species richness is remarkable and comparable with Neotropical floras (Goldblatt and Manning, 2002). The Succulent Karoo biome is the only arid region in the world identified as a biodiversity hotspot. An intriguing feature of the Cape flora is the elevated diversification of a limited number of plant lineages; Linder (2003) demonstrated that 50 % of the species diversity of the CFR can be ascribed to only 33 radiation events. Molecular dating has revealed that, despite considerable variation in the ages of these 33 lineages (Verboom *et al.*, 2009), most modern species of the Cape flora are recent and evolved during the Plio-Pleistocene (Linder and Hardy, 2004).

Several factors promoting rapid diversification in the CFR have been proposed (Linder, 2005), including pollinator specialization (i.e. ethological forces leading to the selection of different floral morphologies), diverse edaphic conditions (i.e. sister species restricted to different soil types) and climatic gradients (i.e. sister species with different climatic requirements). In addition, mechanisms that fragment distribution ranges and lead to geographically isolated populations (e.g. sea-level changes and/or fires) are also believed to have

contributed to this extreme species richness. The combination of complex environmental conditions together with relative long-term climate stability that promoted high speciation and/or low extinction rates has been suggested as the major cause of the present-day hyperdiversity of the Cape flora (Schnitzler *et al.*, 2011). Surprisingly, polyploidization, which is widely recognized as one of the major forces in plant evolution (Soltis *et al.*, 2009), has not been considered as a major driver of radiation in the Greater CFR (Goldblatt and Manning, 2002; Linder and Hardy, 2004; Linder, 2005). Indeed, several species-rich groups such as *Erica* (Ericaceae), *Aspalathus* (Fabaceae) and the Proteaceae include no recorded polyploid species (Goldblatt, 1978; Goldblatt and Johnson, 1979 onwards). The climatic stability of the region has been suggested as an explanation for the paucity of polyploid plants (Dynesius and Jansson, 2000). However, the assumed scarcity of polyploids in the Greater CFR may well be a consequence of the limited karyological research thus far focused on Cape plants. The majority of published chromosome counts are based on single specimens (e.g. Goldblatt and Takei, 1993; Steiner, 1996; Goldblatt and Manning, 2011), which makes the detection of minority cytotypes and/or intraspecific karyological heterogeneity difficult, if not impossible.

The genus *Oxalis* (Oxalidaceae) is an important component of the South African flora that has undergone major radiation in the Greater CFR. The most recent checklist (Dreyer and Makgakga, 2003) recognizes 195 native species (plus nearly 50 varieties), and several novel species have been described in the last decade (e.g. Dreyer *et al.*, 2009; Oberlander *et al.*, 2009*a*). In fact, *Oxalis* is by far the largest geophytic genus in the Greater CFR (Procheş *et al.*, 2006), and the seventh largest genus (119 species according to Goldblatt and Manning, 2000) within the CFR. It is one of the few geophytic eudicot genera that produce true bulbs (Oberlander *et al.*, 2009*b*). All native South African taxa survive unfavourable periods as subterranean bulbs, with growth confined to periods when temperature and moisture regimes offer suitable conditions (usually during winter and spring). Molecular phylogenetic analysis supported the monophyly of South African species and placed their ancestral area within or close to the CFR (Oberlander *et al.*, 2011). In addition, recent studies have confirmed the systematic value of palynology (Dreyer, 1996), distribution patterns and phylogeography (Oberlander *et al.*, 2002, 2012) and ecological characters such as flowering phenology (Dreyer *et al.*, 2006) in Cape *Oxalis* species. Whereas morphological and molecular approaches have been repeatedly used in attempts to elucidate the taxonomy and/or evolutionary history of the group, little is known about the karyological variation of South African *Oxalis*. Heitz (1927) was perhaps the first to list chromosome numbers for eight South African taxa, with a few additional contributions by Yamashita (1935), Warburg (1938), and Dreyer and Johnson (2000). Marks (1956) concluded that CFR members of the genus displayed limited variation in chromosome size and basic chromosome number (mostly $x = 7$), but harboured some polyploid species.

Conventional chromosome number determination is time- and labour-intensive, which precludes analysis of large population samples. However, knowledge of exact chromosome number is not always necessary, as it can be substituted by the estimation of ploidy level, provided that individual DNA ploidy levels are confirmed by chromosome counts. The most reliable current method of ploidy estimation is DNA flow cytometry (Kron *et al.*, 2007; Suda *et al.*, 2007). This high-throughput technique generates large sample sizes that can provide detailed insight into ploidy variation at different spatial and temporal scales, and uncover rare and previously unrecognized cytotypes. Indeed, ploidy screening in a representative number of individuals and populations has resulted in a substantial increase in recognized ploidy-heterogeneous plant species, as well as the number of different cytotypes per species (e.g. Sonnleitner *et al.*, 2010; Trávníček *et al.*, 2011; Husband *et al.*, 2013).

In this study, we employed DNA flow cytometry (FCM), complemented by conventional chromosome counts, to assess ploidy variation in a widespread member (*Oxalis obtusa*) of this important geophytic CFR genus. In particular, we addressed the following questions: (1) What is the ploidy variation of *O. obtusa* across its distribution range (based on representative sampling)? (2) Is this variation geographically and/or ecologically structured? Where is the centre of ploidy-level diversity? (3) Do mixed-ploidy populations occur and, if so, which cytotypes are involved?

MATERIALS AND METHODS

Study species

Oxalis obtusa Jacq. is a distinct (Supplementary Data Fig. S1) and widespread species native to the Western, Northern and partly also Eastern Cape Provinces of South Africa, in addition to a few records from southern Namibia (Dreyer and Makgakga, 2003). The last regional monographer of the genus (Salter, 1944) regarded *O. obtusa* as 'perhaps the commonest and most widespread of the whole genus in South Africa'. The species is well delimited morphologically (irregularly pitted and sharply angled bulb, longest stamens/styles well exserted from the corolla, ovary without calli, and mostly also retrorse hairs on petioles and peduncles), which makes identification easy. *Oxalis obtusa* typically grows abundantly (it is treated as of Least Concern in the Red List of South African Plants; Raimondo *et al.*, 2009), inhabits different vegetation types and spans a wide altitudinal range. Because of its shallowly seated bulbs, the species is easy to collect and thrives in cultivation. No karyological data have been published for this species.

Field sampling

In total, 355 populations were collected in the Greater CFR during 2007–2012, over an area spanning 29°13′–34°44′S and 17°25′–23°59′E (Supplementary Data Fig. S2; see Supplementary Data Table S1 for locality details). We attempted to cover the entire distribution range of the species, according to the data obtained from the SIBIS: SANBI's Integrated Biodiversity Information System (http://sibis.sanbi.org/). In addition, we aimed to sample morphological variation at each

locality. Different stylar morphs (long-, middle- and short-styled) were collected whenever possible to avoid collection of the same genet (i.e. sibling plants originating asexually from bulbils). A total number of 1014 individuals was sampled. Leaf tissue of all samples was silica-dried in the field. In addition, bulbs from 311 populations (=87·6 %) 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). Additional samples (mostly without exact localities) were provided from the collections of the Botanical Garden in Plzeň, Czech Republic, one of which (from Western Cape, near Ceres) was studied in detail due to its unique ploidy level. Herbarium vouchers are kept in the private herbarium of the corresponding author.

Flow cytometry

DNA ploidy levels were inferred from relative fluorescence intensities of 4′,6-diamidino-2-phenylindole (DAPI)-stained nuclei using FCM. Silica-dried samples were processed within 2 months of collection. Leaf tissue from each plant to be analysed was chopped together with an appropriate volume of the internal reference standard using a sharp razor blade 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). *Glycine max* (L.) Merr. 'Polanka', 2C = 2·50 pg (Doležel *et al.*, 2007), served as a primary reference standard (with a similar but not overlapping genome size to that of most samples studied). Hepta- and octoploid *Oxalis* plants were re-analysed using *Bellis perennis* L. as a standard due to their similarities in genome sizes to that of *Glycine*. The crude suspension was filtered through a 42-μm nylon mesh and incubated for 15 min at room temperature. Isolated nuclei were stained with 1 mL of Otto II buffer (0·4 M $Na_2HPO_4.12H_2O$) supplemented with 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 FloMax software, ver. 2·4d (Partec). Fresh leaf tissue from up to three *Oxalis* plants from the same population was analysed together; each plant was re-analysed separately if mixed-ploidy samples were found or if the quality of the histograms was not sufficiently good (i.e. coefficient of variation, CV, of any peak above 5 %). Samples for which only desiccated tissue was available were analysed separately.

Genome sizes in absolute units (pg of DNA) were determined in 35 samples, representing all cytotypes, using propidium iodide FCM. The staining solution consisted of 1 mL of Otto II buffer, β-mercaptoethanol (2 μL mL^{-1}), propidium iodide and RNase A, type IIA, both at a final concentration of 50 μg mL^{-1}. Each sample was measured at least three times on different days and the fluorescence of 5000 particles was recorded using a Partec CyFlow instrument equipped with a green diode-pumped solid state laser (Cobolt Samba, 532 nm, 150 mW output power). If the between-day variation exceeded the 2 % threshold, the most outlying value was discarded and the sample re-analysed.

Chromosome counts

Chromosome numbers were established from actively growing root tip meristems of cultivated plants. In total, 16 individuals representing all fluorescence categories detected by FCM were analysed. Root tip meristems were collected and pre-treated with 0·002 M solution of 8-hydroxyquinoline for 2 h at 4 °C and 2 h at room temperature. Pre-treated samples were fixed in 3 : 1 ethanol/acetic acid mixture at room temperature for several hours and stored at −20 °C until use. Due to the small chromosome size, preparations were made using enzymatic digestion of cell walls with an enzyme cocktail [1 % cellulase Onozuka (Serva, Heidelberg, Germany), 0·4 % pectolyase and 0·4 % cytohelicase (Sigma, Vienna, Austria) in citric buffer; Weiss-Schneeweiss *et al.* (2012)]. Preparations were made in a drop of 45 % acetic acid, cover slips flipped off, and material stained with 2 μg mL^{-1} DAPI dissolved in the mounting antifade medium Vectashield (Vector Laboratories, Burlingame, CA, USA). Preparations were examined with an epifluorescent microscope Axio Imager M2 (Carl Zeiss, Vienna, Austria) and images were captured with a CCD camera using AxioVision software (Carl Zeiss). A minimum of ten complete and unambiguously countable chromosomal spreads were required for chromosome number estimation.

Data analysis

Vegetation, climatic 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), South African Atlas of Climatology and Agrohydrology (Schulze *et al.*, 2008) and the SRTM digital elevation model of South Africa. In total, 28 variables related to soil (11 attributes), precipitation (16 attributes) and temperature (one attribute) were assessed (Supplementary Data Table S2). Climatic parameters with the largest differences among the sites harbouring different majority (i.e. 2*x*, 4*x*, 6*x*) cytotypes were selected by canonical discriminant analysis using SAS 8·1 (SAS Institute, Cary, NC, USA). A generalized linear model (procedure GenMod in SAS 8·1) was used to test whether individual ploidy levels show associations with geographical (latitude, longitude and altitude) and vegetation parameters of sampled localities. The presence/absence of cytotypes in localities fitted a binomial distribution, which was therefore used with the logit link function as parameters of the model.

RESULTS

Ploidy variation

FCM analyses mostly yielded high-resolution histograms, with low background and distinct peaks of both the sample and the internal reference standard. Average sample and standard CVs of G0/G1 fluorescence peaks in DAPI staining were 3·10 % (range 1·46–4·99 %) and 1·96 % (range 1·21–3·32 %), respectively, while in propidium iodide staining were 2·98 % (range 1·86–3·68 %) and 2·02 % (range 1·54–2·77 %), respectively. Importantly, living and desiccated tissues yielded virtually identical DAPI fluorescence intensities (the

TABLE 1. *Results of flow cytometric and karyological analysis of* Oxalis obtusa *samples*

Ploidy level	Fluorescence intensity (mean ± s.d.)*	*n*	2C-value (mean ± s.d.; DNA pg)*	*n*	Chromosome number	*n*
2*x*	0·257 ± 0·010	84	0·69 ± 0·02	15	2*n* = 14	2
3*x*	0·392 ± 0·005	4	1·02	1	2*n* = 21	1
4*x*	0·540 ± 0·028	119	1·37 ± 0·05	7	2*n* = 28	4
5*x*	0·663 ± 0·014	5	1·73	1	2*n* = 35	2
6*x*	0·804 ± 0·033	138	2·03 ± 0·05	6	2*n* = 42	2
7*x*	0·937	1	2·42	1	2*n* = 49	1
8*x*	1·095 ± 0·022	17	2·77 ± 0·02	4	2*n* = 56	4

* *Glycine max* 'Polanka' (2C = 2·50 pg) was used as a standard for diploid to hexaploid *Oxalis* plants while *Bellis perennis* served as a standard for hepta- and octoploids (the estimated values were re-calculated for *Glycine*).

values recorded in dry samples fell within the range of fluorescence intensities observed in fresh samples). The FCM analysis resulted in seven distinct groups of fluorescence intensities, corresponding to 2*x*, 3*x*, 4*x*, 5*x*, 6*x*, 7*x* and 8*x* plants (Table 1, Fig. 1). Chromosome counts performed for selected samples from each FCM category confirmed the estimated DNA ploidy levels and revealed the corresponding number of chromosomes, from 2*n* = 14 to 2*n* = 56, all based on *x* = 7 (Table 1, Fig. 2). Distinct genome size groups were also obtained using propidium iodide FCM (Table 1). Monoploid genome sizes (1Cx-value) of all cytotypes amounted to approx. 0·34 pg.

Of 355 field populations (with usually three individuals collected per population), 343 were ploidy-uniform, while cytotype mixtures were found in 12 populations (3·4 %). The most common cytotypes were hexaploids (observed in 138 localities), followed by tetra- and diploids (119 and 84 localities, respectively); these cytotypes are hereafter called 'majority ploidies'. Octoploids occurred at 17 localities, while rare tri- and pentaploids were only observed at four and five localities, respectively (Fig. 3); these cytotypes are hereafter called 'minority ploidies'. Although we did not find any heptaploid individual *in situ*, this ploidy level was determined in one sample provided by the Plzeň Botanical Garden, CZ (originally collected near Ceres). Mixed-ploidy populations consisted of the following cytotype combinations: di- and triploid (one population), di- and tetraploid (three), di- and pentaploid (one), di- and hexaploid (one), di- and octoploid (one), tri- and hexaploid (one) and tetra- and hexaploid (four).

Cytogeography

Oxalis obtusa mostly occurs in Fynbos, Renosterveld (together comprising the Fynbos biome) and Succulent Karoo vegetation (Figs 3 and 4). The generalized linear model revealed that the distribution of tetraploids and hexaploids is most significantly affected by the vegetation type (Table 2). The majority of tetraploid populations (49·6 %) occurred in Succulent Karoo vegetation while only 15·1 % of 4*x* populations were found in Fynbos vegetation (Fig. 4). Hexaploids exhibited the opposite pattern, with 47·8 and 18·6 % of populations occurring in Fynbos and Succulent Karoo vegetation, respectively. The minority cytotypes (3*x*, 5*x*, 8*x*) were largely restricted to the Fynbos Biome (Fig. 3). The sites inhabited by different majority cytotypes differed mainly in precipitation parameters but only to a limited extent by soil parameters and temperature (Supplementary Data Table S2 and Fig. S4).

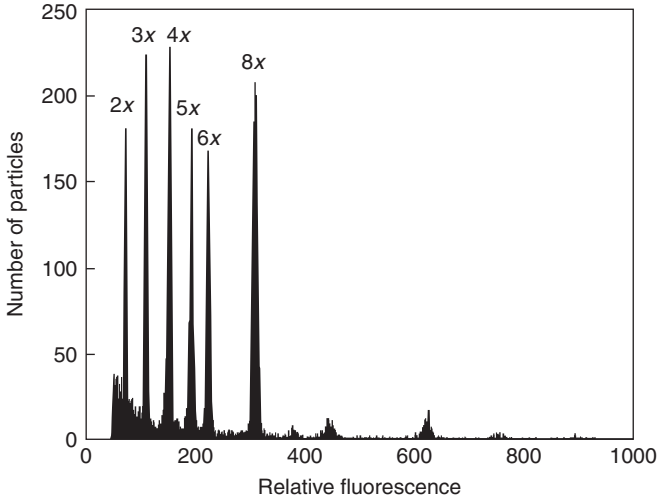

FIG. 1. Fluorescence histogram of simultaneous analysis of six different ploidy levels of *Oxalis obtusa* collected in the Greater Cape Floristic Region. Nuclei from all samples were simultaneously isolated, stained with DAPI and analysed.

Hexaploids are the most widespread cytotype and the only one that extends eastwards of the 22nd meridian (for approx. 200 km further; Fig. 3, Supplementary Data Fig. S3). They are largely absent from interior regions, including the western half of the dry Klein Karoo and the cold Roggeveld. In contrast, octoploids display the most restricted range of all even ploidy levels. Their centre of distribution is located in the extreme south-western Cape, with isolated occurrences in two mountain passes further north/north-east (see Supplementary Data Table S1 for locality details). Diploids prevail along the Atlantic coast and extend the furthest north, with a break in the Knersvlakte (Fig. 3). Tetraploids dominate the interior Roggeveld plateaux and the Klein Karoo, but seem to be (nearly) absent from the south-west Cape. Rare triploids were only observed in the south-west Cape, while pentaploids are more dispersed, and like tetraploids, are mostly absent from the south-west Cape (Fig. 3).

DISCUSSION

This study represents the most comprehensive investigation of ploidy variation in any plant species native to the Greater CFR.

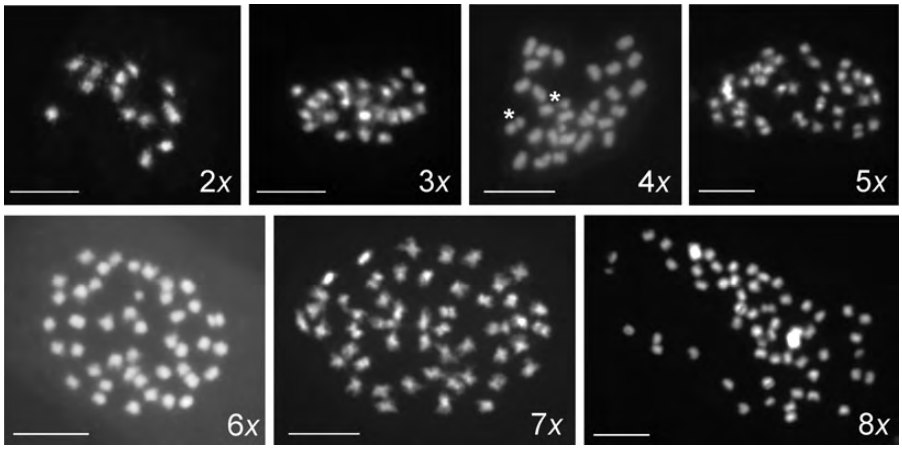

FIG. 2. Mitotic metaphase chromosomes of seven different cytotypes of *Oxalis obtusa.* Asterisks indicate chromosomes with interstitially localized secondary constriction (NOR). NOR is typically decondensed in metaphase chromosome and due to physical force applied during squashing the two parts of NOR-chromosome might detach, thus influencing the correct interpretation of chromosome number. Scale bars = 5 μm.

We found a surprising range of ploidy levels in this widespread member of the geophytic genus *Oxalis*. The distribution of cytotypes was also found to be associated with vegetation types, climatic parameters and geography.

The state of karyological knowledge of the Cape flora

The Cape flora has been largely neglected karyologically, for several possible reasons. First, no or only limited ploidy variation has been found in the few groups subjected to karyological investigation, including speciose genera such as *Gladiolus* and other Iridaceae, *Erica*, *Leucadendron*, *Protea*, *Wahlenbergia*, etc. (Smith, 1992; Goldblatt, 1995; Goldblatt and Takei, 1997; see also Goldblatt and Johnson, 1979 onwards). The claimed lack of polyploidy may have deterred researchers from conducting more extensive cytological research, and more attention was focused on other, particularly molecular, approaches. Considering the long-term relative climate stability in the CFR, which has not supported large-scale plant migrations and subsequent interactions between different lineages/species possibly leading to genome duplication, the lack of polyploids seemed to be a logical conclusion (Dynesius and Jansson, 2000). In addition, the life form composition, such as the predominance of woody species (more than 55 % according to Goldblatt and Manning, 2002) and the abundance of monocotyledonous geophytes with large genome sizes, also did not seem to imply a high incidence of polyploidy. Last but not least, conventional chromosome cytology is time- and labour-intensive and thus not particularly suited for large population studies or very large genera such as those of the Cape flora.

Intraspecific ploidy variation in Oxalis obtusa

By analysing more than 350 populations and usually multiple individuals per population (over 1000 individuals in total), we showed that *O. obtusa* displays remarkable ploidy-level diversity, unlike any other cytologically known Greater CFR plant (cf. Goldblatt and Johnson, 1997 onwards). In total, six different cytotypes (2*x*, 3*x*, 4*x*, 5*x*, 6*x* and 8*x*) were recorded *in situ*, in addition to one heptaploid individual cultivated in the botanical garden in the Czech Republic. To the best of our knowledge, the highest previously known number of ploidy levels in any Cape species was three, found in *Berzelia intermedia* (D.Dietr.) Schltdl., Bruniaceae (Goldblatt, 1981) and *Diascia capsularis* Benth., Scrophulariaceae (Steiner, 1996). Both of these species include di-, tetra- and hexaploid cytotypes.

There are also previous reports of the incidence of two or even three different ploidy levels in some South African species of *Oxalis* (e.g. Heitz, 1927; Marks, 1956). However, caution should be exercised when interpreting old karyological counts, due both to taxonomic (i.e. misidentifications) and to methodological problems. The chromosomes of *O. obtusa* were found to be small, and cell divisions in root meristems were limited. In addition, one (in diploids) to several (in polyploids) chromosome pairs with interstitially localized nucleolar organizing regions (NORs; secondary constrictions) can be present in the complement (Fig. 2). Chromosomes with these secondary constrictions easily break into two parts when specimens are squashed during slide preparation. This is caused by the lower degree of chromatin condensation in the NOR in metaphase chromosomes. Thus, the correct interpretation of chromosome numbers has to be carefully confirmed with multiple chromosome spreads, and some published older aneuploid counts should be treated with caution.

The level of ploidy polymorphism found in *O. obtusa* is very uncommon even at a global scale. Six different ploidy levels have been recorded in both *Ixeris nakazonei* (Kitam.) Kitam. (Asteraceae) from the Ryukyu Archipelago and Taiwan (Denda and Yokota, 2004) and *Cardamine yezoensis* Maxim. (Brassicaceae) from Japan (Marhold *et al.*, 2010). Perhaps the most salient case of intraspecific ploidy variation ever published is the European Alpine *Senecio carniolicus* Willd. (Asteraceae) with eight different cytotypes (2*x*–9*x*; Sonnleitner *et al.*, 2010). This species was long considered to be uniformly hexaploid, and only representative ploidy screening using a combination of FCM and conventional chromosomal

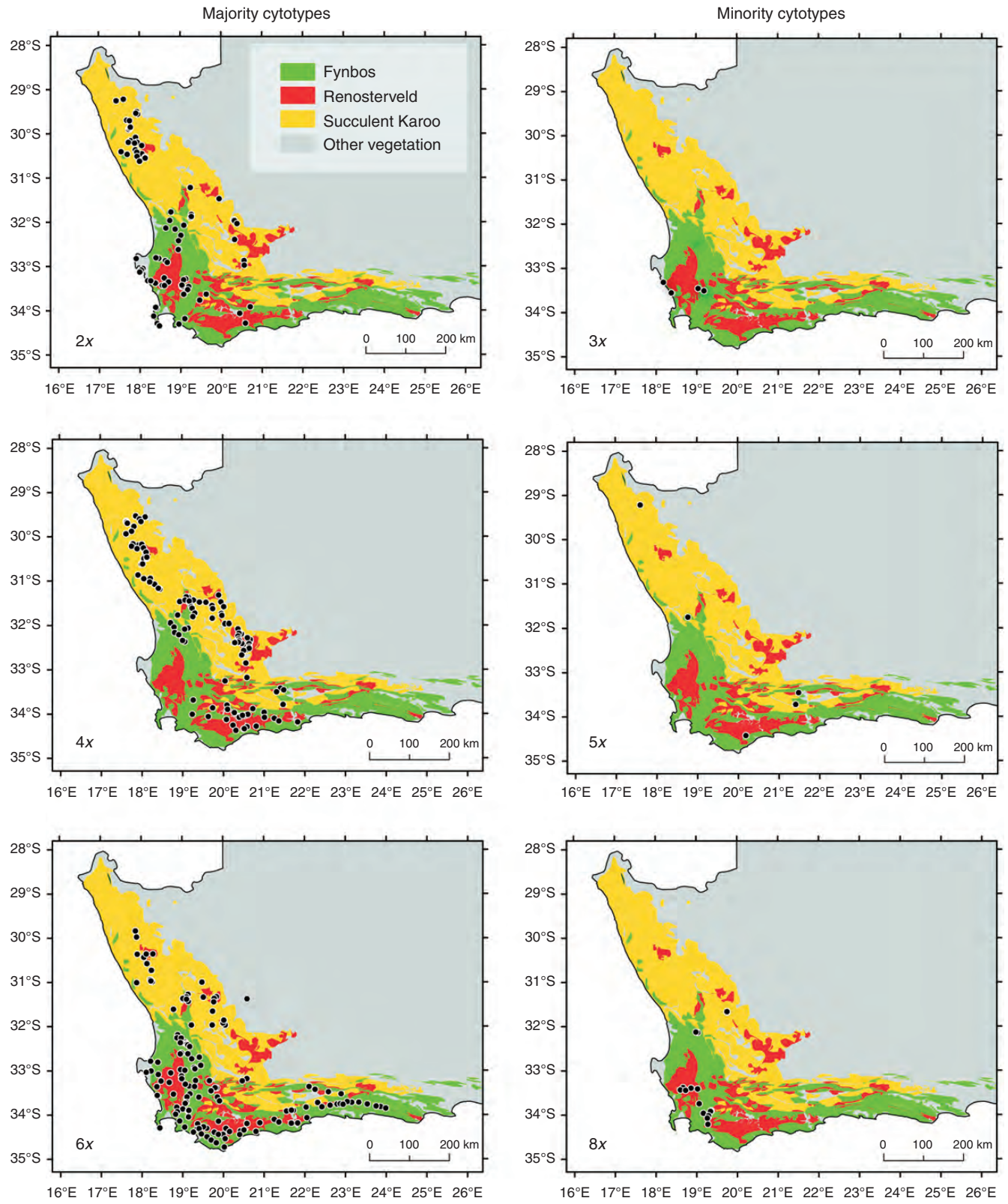

FIG. 3. Distribution of six different cytotypes of *Oxalis obtusa* in the Greater Cape Floristic Region. Three major vegetation types (Fynbos, Renosterveld, Succulent Karoo) are displayed.

counts revealed its karyological complexity. A similar scenario emerged in *O. obtusa*, which had no previous karyological records, but became the most variable Cape species with respect to the number of cytotypes after our thorough FCM study. It is therefore possible that detailed investigation of other species and/or genera in the Cape flora will also uncover a much higher incidence of polyploidy than currently recognized.

The phenotypic similarities of the different ploidy levels (Supplementary Data Fig. S1) together with nearly identical monoploid genome sizes (Cx-values) strongly suggest that polyploids in *O. obtusa* are of autopolyploid origin. This is in agreement with the near-complete lack of interspecific hybrids reported for South African *Oxalis* (Salter, 1944). In addition, mean Cx-values for the closest relatives of *O. obtusa* (Oberlander *et al.*, 2011) differ by more than 10 % from those of *O. obtusa* (J. Krejčíková *et al.*, unpubl. res.). *Oxalis obtusa* is one of the most distinctive South African *Oxalis* species, which excludes taxonomic misidentification as a potential explanation for the exceptional karyological diversity detected in this study. It is likely that both the production of unreduced gametes and inter-ploidy crosses contributed to the ploidy level complexity of *O. obtusa*, and that same-ploidy individuals may have originated via different evolutionary pathways. Putative inter-cytotype mating interactions that underpin the observed ploidy diversity are summarized in Supplementary Data Fig. S5.

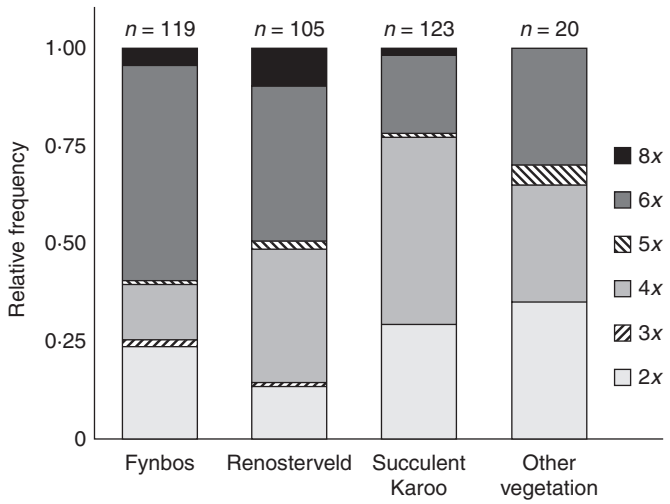

FIG. 4. Relative frequency of different cytotypes of *Oxalis obtusa* in the major vegetation types of the Greater Cape Floristic Region.

Cytogeography

Although the overall picture of ploidy-level distribution in *O. obtusa* is quite complex, some spatial patterns can be discerned (Figs 3 and 4). Following the regional centres of endemism recognized within the CFR (Goldblatt and Manning, 2002), the highest ploidy diversity is concentrated in the Southwestern Centre, which hosts all known triploid populations and the majority of octoploid populations, in addition to the three even-ploidy cytotypes. In contrast, only hexaploids occur in the Southeast Centre. Localities of minority ploidies ($3x$, $5x$, $8x$) were largely (approx. 85 % of sites) situated in Fynbos vegetation, emphasizing the central role of this vegetation type in preserving Cape plant biodiversity. Despite an overlap in geographical ranges of different majority cytotypes, our analyses revealed ploidy sorting according to vegetation and climatic parameters, and geographical location. In particular, hexaploid abundance in different vegetation types decreased in the direction Fynbos → Renosterveld → Succulent Karoo, whereas the opposite was true for tetraploids (Fig. 4). Because the vegetation composition in the Greater CFR is largely affected by rainfall patterns (Mucina and Rutherford, 2006), it is perhaps not surprising that the amounts of precipitation (both total and monthly) were identified as the most important abiotic parameters associated with ploidy distribution in *O. obtusa*. Association between cytotype and vegetation type has been recorded in, for example, *Allium oleraceum* L., Amaryllidaceae (Duchoslav *et al.*, 2010) and *Senecio carniolicus* (Sonnleitner *et al.*, 2010). Similarly, the distribution of diploids and allotetraploids in *Brachypodium distachyon* (L.) P.Beauv., Poaceae, was geographically structured according to an aridity gradient (Manzaneda *et al.*, 2012).

Despite rather limited sampling per locality (mostly three individuals), we were able to detect several (12 out of 355) mixed-ploidy populations. In total, seven different ploidy combinations were observed, involving all three possible combinations of majority cytotypes (i.e. $2x + 4x$, $2x + 6x$, $4x + 6x$) and four further combinations of one majority plus one

TABLE 2. *Associations between the presence of three majority cytotypes of* Oxalis obtusa *and vegetation and geographical parameters of the sites (and their interactions) as tested using the generalized linear model*

		Diploids		Tetraploids		Hexaploids	
Variable	d.f.	χ^2	*P*	χ^2	*P*	χ^2	*P*
Vegetation	2	5·83	0·0543	33·77	**< 0·0001**	39·44	**< 0·0001**
Latitude	1	6·56	**0·0105**	1·94	0·1633	14·08	**0·0002**
Longitude	1	29·45	**< 0·0001**	10·83	**0·0010**	1·31	0·2517
Altitude	1	1·67	0·1959	1·03	0·3108	0·54	0·4635
Latitude × longitude	1	0·70	0·4018	3·38	0·0661	1·90	0·1683
Latitude × altitude	1	1·30	0·2541	1·52	0·2173	2·91	0·0879
Longitude × altitude	1	5·13	**0·0235**	3·96	**0·0466**	1·34	0·2462
Latitude × longitude × altitude	1	2·04	0·1537	9·02	**0·0027**	2·12	0·1450

Significant values ($P < 0·05$) are highlighted in bold.

minority cytotype ($2x + 3x$, $2x + 5x$, $2x + 8x$, $3x + 6x$). As large single-cytotype areas are uncommon in *O. obtusa* (tetraploids in the Sutherland area and hexaploids in the western Agulhas Plains, Ceres region and eastern part of the distribution range are prominent exceptions; see Fig. 3), more representative sampling would probably reveal higher incidence of mixed-ploidy populations. The number of known populations with ploidy-level heterogeneity has increased dramatically during the last decade due to FCM; the sympatric occurrence of up to five different cytotypes is currently known (Trávníček *et al.*, 2011).

Concluding remarks

It would be premature to make any generalizations regarding the role of genome duplication in the genesis of hyperdiversity in the Cape flora. Nonetheless, our pioneering cytogeographical study performed in the Greater CFR strongly suggests that ploidy-level variation in this region can be much higher than currently assumed and that genome-wide processes (initiated by polyploidy) may have significantly contributed to shaping the diversity of the native plant biota. Further research in other plant groups, and on other *Oxalis* species, is crucial to determine whether the observed ploidy polymorphism in *O. obtusa* is an exception or whether polyploidization should be considered as an important, yet neglected, evolutionary driver in this biodiversity hotspot.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Fig. S1: images of different cytotypes of *O. obtusa*. Fig. S2: geographical location of 355 sampling sites of *O. obtusa*. Fig. S3: box plots of geographical characteristics of sites inhabited by the three majority cytotypes of *O. obtusa*. Fig. S4: canonical discriminant analysis of 345 populations inhabited by the three majority cytotypes. Fig. S5: putative origins and mating interactions of six different cytotypes of *O. obtusa* recorded *in situ*. Table S1: locality details of 355 field populations and one *ex situ* population of *O. obtusa*. Table S2: relative contributions of selected abiotic parameters to the canonical axes.

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