



RESEARCH ARTICLE

Morphological and habitat differentiation between diploids and tetraploids of a Drakensberg near-endemic taxon, *Rhodohypoxis baurii* var. *platypetala* (Hypoxidaceae)

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Abstract

Environmental factors may shape the spatial distribution of ploidy levels. Here, we undertook a cytogeographical study of *Rhodohypoxis baurii* var. *platypetala* (Hypoxidaceae), a Drakensberg near-endemic taxon. We addressed the following questions: (1) Are there mixed-ploidy populations or is each population represented by a single ploidy level? (2) Is there a pattern in the environmental distribution of ploidy levels? (3) Are there specific environmental variables associated with each ploidy level locality? (4) Are plant traits similar or different within and among ploidy levels across populations that experience different environmental factors? We measured leaf and flower traits of individuals that were sampled for flow cytometry from 17 populations across the KwaZulu-Natal and Free State provinces in South Africa. We extracted daily climate data for 13 variables and collected soil samples to evaluate pH and nutrient properties to characterize the sampled populations to test for relationships with ploidy level distributions. Twelve populations were found to contain only diploids, four populations contained only tetraploids, and only one population was 'mixed ploidy' (both diploid and triploid individuals present). There was an overlap in the altitudinal range of diploid and tetraploid populations, but diploids reached the highest altitudes recorded for the current study. We also found that *R. baurii* var. *platypetala* occurs in acidic soils and that tetraploids occurred in soils with marginally higher nitrogen and phosphorus than soils where diploids occur. Tetraploids generally occurred in warmer conditions, in drier soils, and possessed broader leaves and larger flowers than diploids. Our study suggests that soil factors and temperature at a small (within localities) spatial scale likely shape ploidy level distributions in the Drakensberg grasslands.

KEYWORDS

cytogeography, flow cytometry, Hypoxidaceae, mountain grasslands endemic, temperature

INTRODUCTION

Polyploidy, where organisms possess more than two complete sets of chromosomes in the nuclei (de Wet, 1971; Otto & Whitton, 2000; Ramsey & Schemske, 1998), is widely considered an important mechanism of evolution in angiosperms (Ramsey & Ramsey, 2014; Weiss-Schneeweiss et al., 2013; Wood et al., 2009). Polyploid plants are globally distributed, and

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environmental factors such as annual mean temperature and latitude have been suggested as key drivers of this global distribution (Bureš et al., 2024; Rice et al., 2019). These variables are likely influencing range boundaries of different ploidy levels. Previous work also correlated polyploid distribution with environmental stress or change (reviewed by Van der Peer et al., 2017). However, work at the global level may not necessarily be informative for predicting polyploid distribution patterns at a local scale. Currently, there are inconsistent trends in local-scale ploidy level distribution patterns for plant species in different hemispheres. For instance, in North America, while climatic niche differences have been found between diploids and polyploids and they were best explained by a shift in precipitation availability in some species, with diploids occurring in drier conditions than polyploids (e.g., *Tolmeia* (Saxifragaceae), Visger et al., 2016), in other species, this pattern was reversed as polyploid populations occupied drier habitats than diploid populations (e.g., *Chamerion angustifolium* (Onagraceae), Thompson et al., 2014). In the Cape Floristic Region of South Africa, the distribution of the different ploidy levels of *Oxalis obtusa* (Oxalidaceae) showed evidence for ploidy co-occurrence (Krejčíková et al., 2013), and in *Dicerotheramnus rhinocerotis* (Asteraceae), high niche equivalency and niche similarity was found for diploids and tetraploids (Chumová et al., 2023), suggesting that a prevalent pattern of niche shifts may not always exist between diploids and polyploids (Glennon et al., 2014).

Local-scale distribution patterns of ploidy levels (e.g., Afonso et al., 2021; Castro et al., 2018, 2019; Husband & Schemske, 1998) may be governed by the putative competitive advantage polyploids have over diploids in stressful environmental conditions like drought, heat stress, and salinity (Chao et al., 2013; Godfree et al., 2017; Liu et al., 2011; Maherali et al., 2009; Yang et al., 2014; Zhang et al., 2010; but see Mtileni et al., 2021; Wei et al., 2020). Further, polyploids may be more frequent in disturbed and vacant habitats, where there may be niche availability and lower competition (e.g., Kiedrzyński et al., 2021). If niches are available and competition is low, these advantages may enable polyploids to occupy a geographically broader distribution range than diploids (e.g., Kiedrzyński et al., 2021). The availability of nitrogen and phosphorus may also affect polyploid distributions, due to the higher demands of nitrogen and phosphorus by the nucleus after whole genome duplication (Faizullah et al., 2021; Leitch & Leitch, 2008). There is a clear need for more work focusing on the local-scale factors that shape ploidy level distributions and maintenance of their range boundaries in natural populations.

While niche divergence between polyploids and their diploid progenitors is possible (Thompson et al., 2014; Visger et al., 2016), there is also evidence for shared or nested environmental niches of diploids and polyploids (Castro et al., 2019; Glennon et al., 2014). This discordance in broad-scale patterns may be a result of how polyploids respond to environmental variables in different regions or at smaller scales. For example, polyploids may be more successful than diploids in drier conditions (Maherali et al., 2009) and under intense solar radiation (Zozomová-Lihová et al., 2015). There is a limited understanding of polyploidy in the southern hemisphere mountain grasslands, especially in the Drakensberg grassland ecosystem (but see work on the geographically close but floristically dissimilar Cape Floristic Region such as Chumová et al., 2023; Elliot et al., 2023; Krejčíková et al., 2013; Veselý et al., 2020, where genome size and ploidy levels have been associated with plant phenotypes and environmental correlates). The intraspecific ploidy variation in *Rhodohypoxis baurii* var. *platypetala* (Hypoxidaceae; Saito, 1975) presents an opportunity to examine the distribution of ploidy levels in their natural environments. We investigated whether populations of *R. baurii* var. *platypetala* comprised one or more

ploidy levels, assessed whether ploidy levels are evenly distributed across the Drakensberg grassland region, and then tested whether there were environmental differences between diploid and tetraploid populations to infer how these variables may have shaped patterns of ploidy level distributions in these natural populations. Lastly, we measured morphological traits to test if traits remained consistent across populations and putative ploidy levels.

METHODS

Study system

Rhodohypoxis (Asparagales: Hypoxidaceae), a Drakensberg near-endemic plant genus, comprises six species of herbaceous perennial geophytes that occur in the mountain grasslands of the KwaZulu-Natal Drakensberg mountains in South Africa, potentially into the Barberton region of Eswatini, and along the eastern edges of the Lesotho plateau (Hilliard & Burt, 1978). The genus-level taxonomy is contentious, so we are following the taxonomic designation of Hilliard and Burt (1978) for the current study. For this study, we focused on *R. baurii* var. *platypetala*, the most widespread variety ('taxon' hereafter) of *R. baurii*, which occurs in grass patches, in dry, shallow, stony soils over rock sheets, and produces broad, grey-green leaves, and predominantly white (sometimes light pink), relatively flat flowers with six tepals (Hilliard & Burt, 1978). This taxon is widespread across the KwaZulu-Natal province in South Africa, with a few small populations that are geographically close in the Free State province along the northern Drakensberg escarpment and in the Eastern Cape province, also along the Drakensberg escarpment. Earlier work showed chromosome squash evidence for at least two ploidy levels (diploids and triploids) in this taxon (Saito, 1975), and this potential intraspecific ploidy variation led us to examine whether different ploidy levels might exist in natural populations.

Leaf sample collection in the field

Seventeen naturally occurring populations of *R. baurii* var. *platypetala* were identified across the Free State and KwaZulu-Natal provinces in South Africa. All populations occurred on relatively dry soils with patchy grasses and shallow stony soils over rock sheets as described by Hilliard and Burt (1978). Leaf sample collections for flow cytometry were carried out in the peak growing season for *R. baurii* var. *platypetala* (November) over 2 years (2021 and 2022). For each population, we sampled 30 individuals (per permit regulation), or slightly over 30 individuals for large populations occurring on private farms, and individuals were sampled only if they possessed more than three leaves. From these individuals, fresh leaf samples (two to three leaves per individual) were collected after leaf and flower measurements were taken (see below). In some instances, we sampled fewer than 30 individuals due to small population size. Individual plants were sampled systematically by placing a 1 m squared quadrat in each population six times and sampling five individuals inside the quadrat (one from each corner and one in the centre). Quadrats were positioned at least 3 m apart to minimize sampling the same individuals and to cover the extent of the population. For small populations that comprised fewer than 100 plants, or where individuals grew near each other or in between rocks, we did not use quadrats but ensured that the sampled individuals were spaced at least 3 m apart to cover the extent of the population. Leaves from each

individual plant were placed in separate moistened paper towels within plastic bags. All leaf samples were labelled and placed in a cooler box with ice packs and kept cool until flow cytometry analyses could be completed.

Quantifying field plant size traits

In 2021 (November), we measured leaf and flower traits of *R. baurii* var. *platypetala* plants from nine of the 17 sampled populations to test if there was morphological differentiation between diploids and polyploids (Table 1). These populations were chosen due to accessibility and time allocated for sampling in private property. We measured the following traits on 270 plants: number of leaves, number of flowers and buds, largest-leaf width (mm), largest-leaf length (mm), stem height (mm) of the largest flower, and the largest-flower length (mm) and width (mm) of the outer tepals. Plant size was calculated as the product of the number of leaves and largest-leaf width. Outer tepal size of the largest-flower per each individual was calculated as the product of the largest-flower length and corresponding width of the outer tepals. All measurements were taken before harvesting leaf tissue samples for flow cytometry – i.e., without knowing the ploidy levels of the measured individuals, to avoid sampling bias. Flow cytometry results later showed that each of the nine sampled populations comprised largely a single ploidy (five diploid, four tetraploid). Based on our preliminary results from flow cytometric analyses on plants that were collected from the mixed-ploidy population and housed in the greenhouse, we had intended to measure traits on triploid individuals, but our sampling yielded only seven triploid individuals from this population (Table 1). Generalized linear models were constructed using the base *stats* package (R version 4.2.1; R

TABLE 1 Ploidy level and altitude (m.a.s.l.) of *Rhodohypoxis baurii* var. *platypetala* populations that were sampled for this study.

Population ID	Population locality	Ploidy level (n)	Altitude (m.a.s.l.)
1*	Golden Gate Highlands National Park, FS	2x (31)	2060
2*	Highmoor Nature Reserve, KZN	2x (16)	1940
3*	Drakensberg Gardens, KZN	2x (14)	1830
4	Lower Lotheni road, KZN	2x (16)	1820
5*	Giants Castle Game Reserve (Monks Cowl), KZN	2x (23)	1780
6*	Mt Gilboa Nature Reserve, KZN	4x (26)	1750
7	Underberg, KZN	2x (8)	1750
8	Bushman's Nek, KZN	2x (18)	1750
9*	Cobham Nature Reserve, KZN	2x (30)	1690
10	Ingwe local municipality, KZN	2x (18)	1680
11	Sunset Farm, KZN	2x (27), 3x (7)	1650
12*	Hebron Farm road, KZN	4x (21)	1620
13	Underberg, KZN	2x (9)	1530
14	Underberg, KZN	2x (17)	1490
15*	Minerva Nature Reserve, KZN	4x (23)	1490
16	Stoffelton road, Ngqiyana, KZN	2x (20)	1460
17*	Howick, KZN	4x (26)	1240

Note: (n) represents the number of individuals that were analysed for ploidy level. FS refers to Free State province, KZN refers to Kwa-Zulu Natal province. Populations where trait measurements were taken are indicated by an asterisk. Population IDs correspond to the IDs on Figure 2 and Table 5.

Core Team, 2022) to test if ploidy and population had a significant effect on each trait. Further, Kruskal–Wallis tests were used to compare each trait between diploid and tetraploid populations. When a significant effect of both ploidy and population on the traits was found, we used the Pearson correlation coefficient for normally distributed data and the Spearman rank-correlation coefficient for non-normally distributed data to test the correlation between the traits and climatic variables that were extracted from the populations where the traits were measured (see details below). The Holm-Bonferroni adjustment was undertaken on the p -values to control for the possibility of Type I due to multiple comparisons (Holm, 1979). All statistical analyses in this study were conducted in R version 4.2.1 (R Core Team, 2022).

Flow cytometry analyses

Saito (1975) conducted chromosome squashes for *R. baurii* var. *platypetala* individuals that were collected from the Eastern Cape, South Africa, in the early 1970s. Using both herbarium and collection records, we were able to identify the source population used for the squashes (Hebron Farm, Eastern Cape, South Africa). Consequently, we were able to generally associate a flow cytometry measure of plants from the same population to a chromosome count produced by Saito (1975). Flow cytometry was then used to estimate ploidy levels of 350 plants from 17 populations of this taxon (Table 1). Relative fluorescence intensities of 4,6-diamidino-2-phenylindole (DAPI)-stained nuclei were used to infer ploidy levels per Doležel et al. (2007). *Oxalis articulata* (Oxalidaceae; 2C-value ≈ 0.91 pg; Vaio et al., 2014) was used as an appropriate internal reference standard as ongoing work has shown that this standard can be used successfully for *Rhodohypoxis*. Leaf tissue from each individual plant sample and the standard (~ 1 cm² each) were chopped together in 1000 μ L of Otto I (0.1 M citric acid monohydrate, 0.5 mL of Tween 20, and 100 mL of dH₂O) using a new razor blade. The homogenate was filtered into a sample tube through a 30 μ m mesh (Sysmex Partec GmbH, Görlitz, Germany) and then incubated for approximately 20 min at room temperature. Then 1000 μ L of Otto II buffer (0.72 g of Na₂HPO₄·H₂O, 10 mL of dH₂O, 10 μ L of DAPI, and 20 μ L of beta-Mercaptoethanol) was added and the solution was incubated for an additional 10 min at room temperature, before being run on a CyFlow Space (Sysmex Partec GmbH, Görlitz, Germany) flow cytometer housed at the University of Pretoria. FlowMax software (version FloMax 2.9x; Sysmex Partec GmbH, Görlitz, Germany) was used to record the relative nuclei fluorescence of at least 5000 particles, with the same speed (0.5) and gating (50) settings throughout. Samples from the same population were analysed in the same analyses bout. Where possible, samples were rerun to improve their coefficient of variance of the mean fluorescence intensity (CV %) to be below 5% and then used as references for samples from the same population for which improving the CV % was not possible.

Environmental characteristics for sampled populations

Daily climate data, spanning a period of 10 years (2012–2021), were extracted from the National Aeronautics and Space Administration (NASA) Langley Research Centre (LaRC) Prediction of Worldwide Energy Resource (POWER) Project funded through the NASA Earth Science/Applied Science Program (<https://power.larc.nasa.gov/data-access-viewer/>) for the following variables for the 17 populations: (1) temperature at 2 m above the surface

of the Earth ($^{\circ}\text{C}$), (2) dew/frost point at 2 m above the surface of the Earth ($^{\circ}\text{C}$), (3) wet bulb temperature at 2 m above the surface of the Earth ($^{\circ}\text{C}$), (4) Earth skin temperature ($^{\circ}\text{C}$), (5) maximum temperature at 2 m above the surface of the Earth ($^{\circ}\text{C}$), (6) minimum temperature at 2 m above the surface of the Earth ($^{\circ}\text{C}$), (7) all sky surface longwave downward irradiance (horizontal infrared radiation intensity from sky; W m^{-2}), (8) specific humidity at 2 m above the surface of the Earth (g kg^{-1}), (9) relative humidity at 2 m above the surface of the Earth (%), (10) precipitation corrected (mm day^{-1}), (11) surface soil wetness (0–1), (12) root zone soil wetness (0–1), and (13) profile soil moisture (0–1). The zero to one scale indicates a completely water-free soil at a value of 0 and a completely saturated soil at a value of 1. The data were obtained from POWER Project's Hourly 2.0.0 version on 20 March 2023. Kruskal–Wallis tests were used to test if each environmental variable differed between diploid and tetraploid populations and among the diploid, mixed-ploidy, and tetraploid populations. We used a one-way ANOVA to test if altitude differed between the diploid and tetraploid populations.

We then constructed a correlation matrix using the package *corrplot* (version 0.92; Wei & Simko, 2017) to reduce the number of correlated climatic variables by using a correlation coefficient threshold not exceeding 0.60 for all variables (Appendix S5). Finally, four variables were chosen and included in the Principal Component Analysis (PCA) to assess their associations with ploidy level distributions: temperature at 2 m above the surface of the Earth ($^{\circ}\text{C}$), all sky surface longwave downward irradiance (horizontal infrared radiation intensity from sky; W m^{-2}), precipitation corrected (mm day^{-1}), and profile soil moisture (0–1). We also included possible interactions between these variables (see Table 4 for specific details). We formatted the presence of ploidy levels to binary data where diploid population = 0 and tetraploid populations = 1, after which a generalized linear model of the binomial family (link = logit) was used to compare the effect of the four variables on the presence of diploids or tetraploids. We found one mixed-ploidy population containing only seven triploid individuals, so triploids were not included in our analysis.

Soil samples were collected from four populations (three diploid, one tetraploid; Tables 1 and 5) per our collecting permit and landowner permission for analyses of available nitrogen (%; analysed as $\text{NH}_4\text{-N}$), available potassium (mg kg^{-1}) and phosphorus (mg kg^{-1}) contents (analysed using citric acid), and pH. Total nitrogen assessment was not available, so we used reported percentage of available nitrogen in ammonia. We collected two separate soil samples (200 g each) from a depth of up to 10 cm (deeper depths were precluded due to rock sheets, and we observed that *Rhodohypoxis* plant roots rarely go below 10 cm in the soil) into the ground for each population, to ensure consistency in the analyses. All samples were kept in separate, labelled, sealed Ziplock bags and then sent to the Western Cape Department of Agriculture, Elsenburg soil analytical laboratory, Private Bag X1, 7607, South Africa, for analysis.

RESULTS

Flow cytometry analyses

Three ploidy levels were detected in the sampled populations: diploids (Figure 1b), triploids (Figure 1c), and tetraploids (Figure 1d; Appendices S1 and S2), after which four tetraploid populations, 12 diploid populations, and one mixed-ploidy population were identified (Table 1; Figure 2). The mixed-ploidy population was predominantly diploid, with only seven of the 34 sampled individuals found to be triploids, and no tetraploids were found.

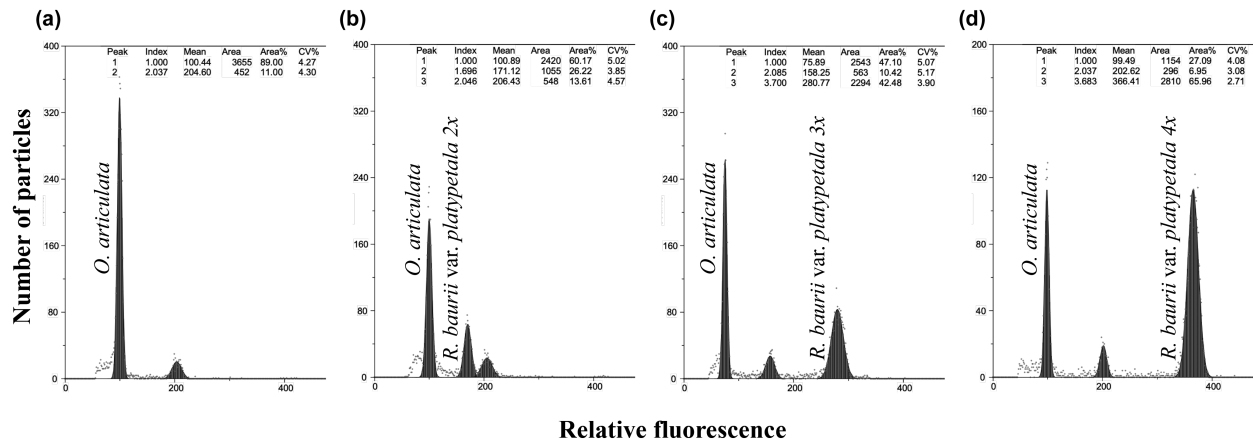


FIGURE 1 Relative fluorescence histograms obtained from flow cytometry analyses of *Rhodohypoxis baurii* var. *platypetala* leaf samples that were used for this study. Ploidy level of the standard, *Oxalis articulata* (a), and three ploidy levels of *R. baurii* var. *platypetala*, diploid (b), triploid (c), and tetraploid (d), are shown.

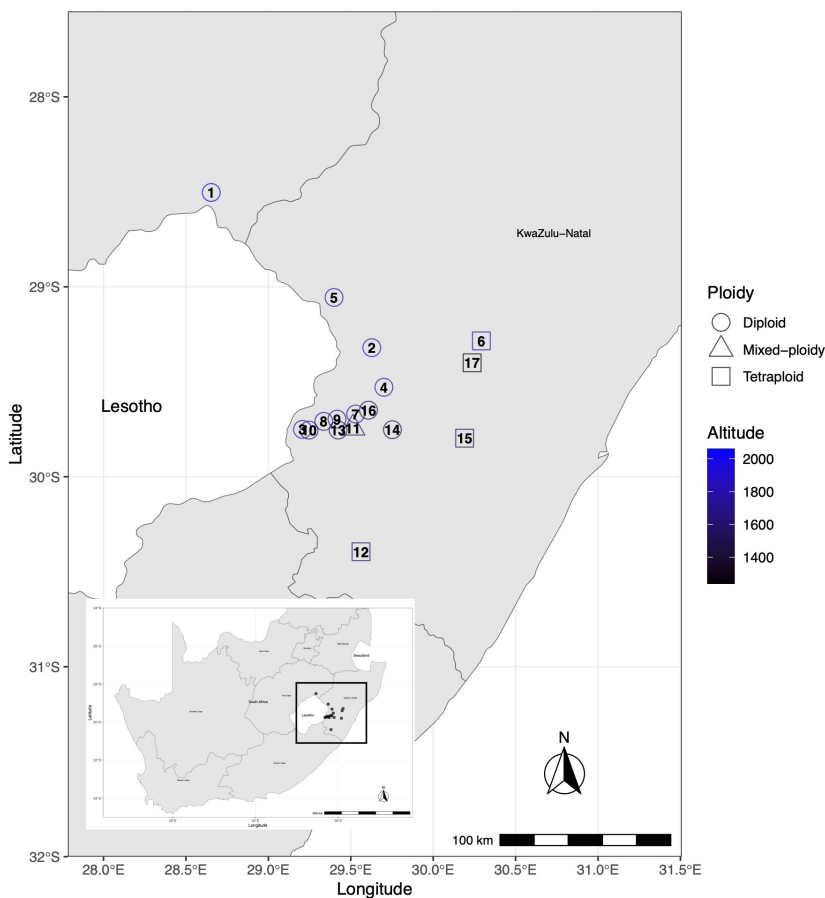


FIGURE 2 A map of 17 sampled populations of *Rhodohypoxis baurii* var. *platypetala* in South Africa, and the distribution of three ploidy levels of this taxon that were identified across the Free State and KwaZulu-Natal parts of the Drakensberg grasslands, South Africa. Circles and squares represent diploid (2x) and tetraploid (4x) populations, respectively, and the mixed-ploidy population (2x, 3x) is represented by a triangle. The colour gradient represents the altitude (m.a.s.l.) where each population occurs. Population IDs correspond to the IDs in Tables 1 and 5.

Geographic distribution of ploidy levels

Diploids occurred between 1460 and 2060 m a.s.l., whereas tetraploids occurred between 1240 and 1750 m a.s.l. (Table 1). While diploids reached the highest recorded altitudes for the current study than tetraploids (Table 1; Figure 2), altitude did not significantly differ between diploid and tetraploid populations ($F(1)=3.65, p=0.08$). The mixed-ploidy population occurred at 1650 m a.s.l. (Table 1). Latitude did not significantly differ between diploid and tetraploid populations ($H(1)=0.37, p=0.54$).

Trait measurements

The number of leaves was significantly affected by ploidy ($p<0.0001$; Table 2), and diploids possessed more leaves than tetraploids (Figure 3). The number of flowers and buds, and largest-leaf length, were not significantly affected by ploidy (Table 2). Tetraploids possessed broader leaves and larger flowers and larger plants than diploids (Figures 3 and 4), but both ploidy and population had a significant effect on these traits (Table 2). We found a significant positive correlation between largest-flower size and both precipitation and infrared radiation intensity while the other traits were not significantly correlated to any variable (Appendix S3).

Environmental characteristics of sampled populations

Daily data of all 13 climatic variables differed significantly between the diploid and tetraploid populations (for 13 different models; $p<0.0001$), and overall, the variables' means were higher for tetraploid populations compared to diploid populations (Table 3). Tetraploids generally occurred in warmer and more humid conditions with increased infrared

TABLE 2 Summary results from generalized linear models of the effect of ploidy (diploids and tetraploids) and population on the number of leaves, number of flowers and buds, largest-leaf length (mm) and width (mm), plant size (mm), largest-flower stem height (mm), and average outer-petal size of the largest flower (mm) of *Rhodohypoxis baurii* var. *platypetala*.

Source	Estimate	SE	t value	p	Estimate	SE	t value	p
(a) Number of leaves					(b) Number of flowers and buds			
(Intercept)	5.69	0.20	29.07	0.0001	2.10	0.17	17.49	0.0001
Ploidy	-0.88	0.26	-3.35	0.001	-0.29	0.23	-1.26	0.21
Population	0.07	0.05	1.35	0.18	-0.14	0.04	-3.19	0.001
(c) Largest-leaf length					(d) Largest-leaf width			
(Intercept)	44.47	2.33	19.05	0.0001	4.99	0.27	18.34	0.0001
Ploidy	-4.14	3.14	-1.32	0.19	3.82	0.37	10.44	0.0001
Population	0.45	0.60	0.74	0.46	-0.31	0.07	-4.43	0.0001
(e) Plant size					(f) Largest-flower stem height			
(Intercept)	25.89	1.56	16.56	0.0001	47.08	2.30	20.47	0.0001
Ploidy	13.03	2.10	6.20	0.0001	-5.24	3.09	-1.69	0.09
Population	-0.76	0.40	-1.89	0.06	1.59	0.59	2.67	0.01
(g) Largest-flower tepal size								
(Intercept)	79.71	6.55	12.35	0.0001				
Ploidy	37.6	8.67	4.34	0.0001				
Population	9.38	1.67	5.62	0.0001				

Note: Significant results are bolded, where 0.0001 represents p values that are extremely small to report.

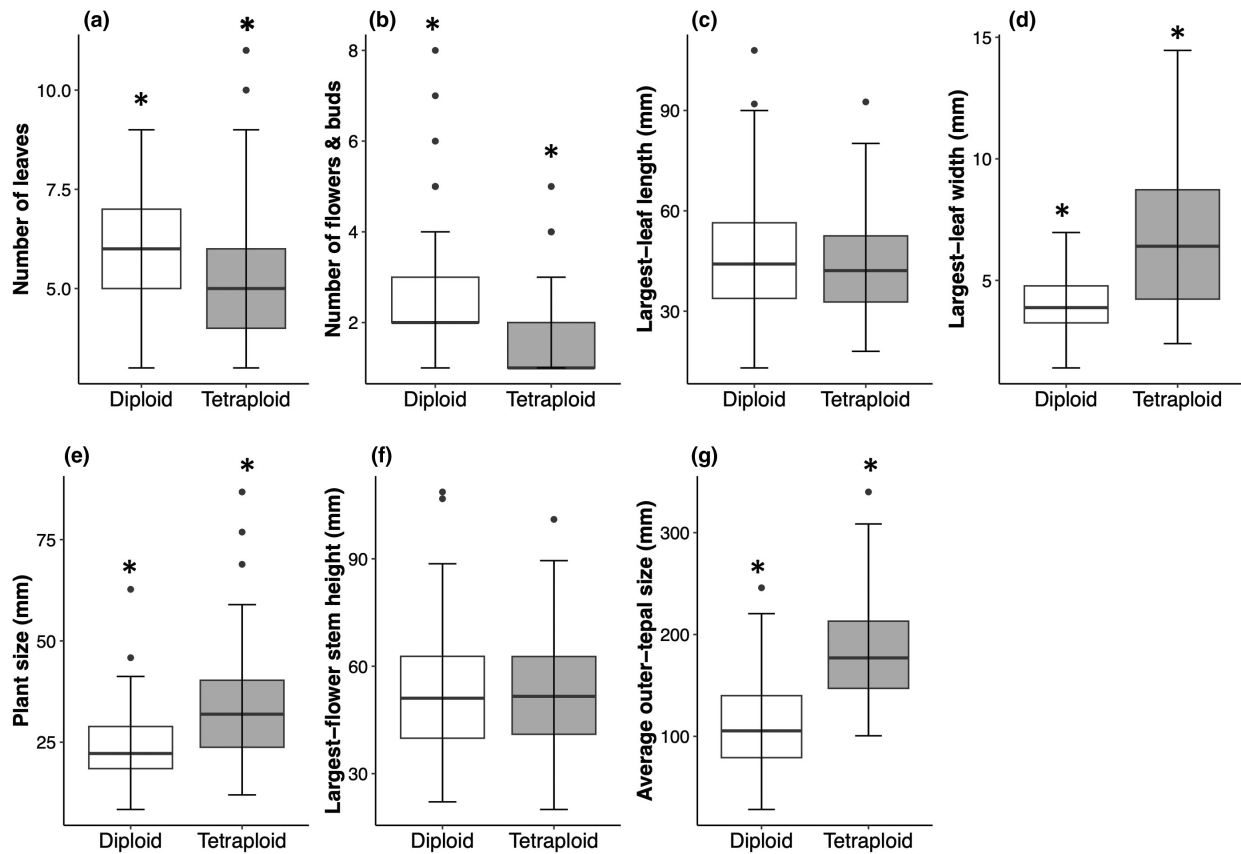


FIGURE 3 Plant size measurements of *Rhodohypoxis baurii* var. *platypetala* diploid (white bars) and tetraploid (grey bars) individuals according to the number of leaves, number of flowers and buds, largest-leaf length (mm) and width (mm), plant size (mm), largest-flower stem height (mm), and average outer-tepal size of the largest flower (mm). The solid lines within boxes represent the median and the ends of the boxes indicate upper and lower quartiles, Whiskers show the highest and lowest values excluding the outliers which are represented by black dots. Asterisks indicate significant trait differences at $p=0.0001$ between diploid and tetraploid populations.

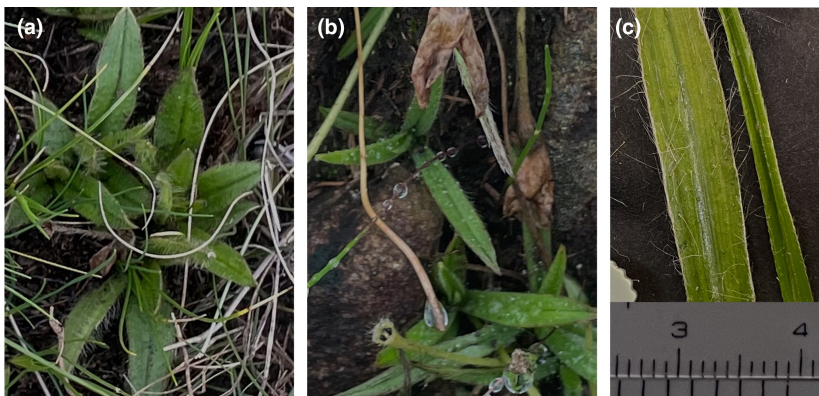


FIGURE 4 Tetraploid (a) and diploid (b) individuals of *Rhodohypoxis baurii* var. *platypetala* in their natural populations, and a comparison of leaf width (cm; c) between tetraploid (left) and diploid (right) individuals.

radiation intensity than diploids (Table 3). Tetraploid populations also experienced higher daily mean precipitation than diploid populations (diploid pop mean = 2.83 mm day^{-1} , tetraploid pop mean = 3.14 mm day^{-1} ; $H(1) = 69.38$, $p < 0.0001$), but tetraploids generally occurred in environments with lower daily mean surface soil wetness, root zone soil wetness, and profile soil moisture than diploids (Table 3). The mixed-ploidy and

TABLE 3 Mean, minimum (min.), and maximum (max.) values \pm SE of 13 climatic variables compared between diploid (2x) and tetraploid (4x) populations of *Rhodohypoxis baurii* var. *platypetala*.

Variable	Min. \pm SE 2x	4x	Mean \pm SE 2x	4x	Max. \pm SE 2x	4x
Temperature at 2 m (°C)	-4.28 \pm 0.90	0.82 \pm 0.67	12.92 \pm 0.50 ^a	15.39 \pm 0.19 ^b	25.51 \pm 0.44	27.58 \pm 0.26
Dew/frost point at 2 m (°C)	-13.24 \pm 0.81	-9.03 \pm 0.69	4.49 \pm 0.65 ^a	8.10 \pm 0.57 ^b	16.15 \pm 0.52	19.19 \pm 0.34
Wet bulb temperature at 2 m (°C)	-6.22 \pm 0.64	-2.45 \pm 0.40	8.71 \pm 0.57 ^a	11.74 \pm 0.37 ^b	18.65 \pm 0.55	21.56 \pm 0.22
Earth skin temperature (°C)	-7.92 \pm 1.44	0.52 \pm 0.70	13.10 \pm 0.48 ^a	15.49 \pm 0.11 ^b	27.07 \pm 0.41	28.62 \pm 0.48
Temperature at 2 m maximum (°C)	1.80 \pm 0.69	5.96 \pm 0.63	20.98 \pm 0.50 ^a	23.32 \pm 0.23 ^b	35.79 \pm 0.66	38.72 \pm 0.16
Temperature at 2 m minimum (°C)	-9.67 \pm 0.83	-4.92 \pm 0.80	6.59 \pm 0.56 ^a	9.46 \pm 0.38 ^b	17.71 \pm 0.60	20.29 \pm 0.18
Longwave downward irradiance (W m ⁻²)	207.47 \pm 1.08	237.61 \pm 5.07	310.40 \pm 0.09 ^a	339.01 \pm 6.05 ^b	389.63 \pm 0.62	412.76 \pm 2.60
Specific humidity at 2 m (g kg ⁻¹)	1.65 \pm 0.10	2.19 \pm 0.09	7.14 \pm 0.23 ^a	8.39 \pm 0.24 ^b	14.26 \pm 0.32	16.17 \pm 0.24
Relative humidity at 2 m (%)	20.37 \pm 0.68	22.75 \pm 1.08	63.31 \pm 0.93 ^a	67.82 \pm 1.48 ^b	97.51 \pm 0.21	97.53 \pm 0.63
Precipitation corrected (mm day ⁻¹)	0	0	2.83 \pm 0.11 ^a	3.14 \pm 0.26 ^b	75.59 \pm 8.73	118.81 \pm 11.87
Surface soil wetness (0–1)	0.28 \pm 0.01	0.29 \pm 0.03	0.60 \pm 0.01 ^a	0.58 \pm 0.02 ^b	0.94 \pm 0.01	0.91 \pm 0.01
Root zone soil wetness (0–1)	0.51 \pm 0.01	0.48 \pm 0.02	0.62 \pm 0.01 ^a	0.58 \pm 0.02 ^b	0.90 \pm 0.02	0.81 \pm 0.04
Profile soil moisture (0–1)	0.51 \pm 0.01	0.49 \pm 0.02	0.62 \pm 0.01 ^a	0.59 \pm 0.02 ^b	0.87 \pm 0.03	0.80 \pm 0.05

Note: Different letters within rows of each mean variable denote significant differences at $p = 0.0001$ between the populations.

diploid populations were similar to each other in daily mean precipitation, relative humidity, and infrared radiation intensity, while these three variables were significantly higher in tetraploid populations (Appendix S4). The first two principal components (PC1 and PC2) cumulatively explained 85.45% of the total variation in the four uncorrelated climatic variables that were selected (Appendix S7). Tetraploid populations tended to occur in the upper-right and lower-right of the PCA ordination, which were characterized by high mean temperature, precipitation, and infrared radiation intensity and low soil moisture, but there was some overlap between diploid and tetraploid populations (Appendices S6 and S7). Further, increases in infrared radiation intensity and an interaction of precipitation and infrared radiation intensity increased the likelihood of tetraploid presence, while the other variables reduced this likelihood as they increased (Table 4).

Soil pH was similar among the four analysed populations (pH = 4.1–4.3; Table 5). Total available nitrogen content (1.35%) and phosphorus content (89 mg kg⁻¹) were higher in the tetraploid population than the three diploid populations, but the available potassium content for one of the diploid populations found in Underberg, KwaZulu-Natal province (247 mg kg⁻¹), was more similar to that of the tetraploid population (221 mg kg⁻¹) while available potassium contents for the other two diploids sites were below 105 mg kg⁻¹ (Table 5).

TABLE 4 Summary results from a generalized linear model of four climatic variables as predictors for the presence of diploid and tetraploid populations of *Rhodohypoxis baurii* var. *platypetala*.

Variable	Estimate	SE	z Value	p	2.5%	97.5%	Odds ratio
Intercept	-4.21	0.13	-32.46	0.0001	0.01	0.02	0.01
Temperature at 2 m (°C)	-0.02	0.004	-6.49	0.0001	0.97	0.98	0.98
Longwave downward irradiance (W m ⁻²)	0.02	0.0005	53.88	0.0001	1.02	1.03	1.02
Precipitation corrected (mm day ⁻¹)	-3.05	0.07	-44.69	0.0001	0.04	0.05	0.05
Profile soil moisture (0–1)	-6.94	0.18	-37.97	0.0001	0.0007	0.001	0.001
Temperature at 2 m (°C) * Precipitation corrected (mm day ⁻¹)	-0.03	0.001	-34.11	0.0001	0.9	0.97	0.97
Precipitation corrected (mm day ⁻¹) * Profile soil moisture (0–1)	-0.22	0.03	-7.02	0.0001	0.75	0.85	0.80
Longwave downward irradiance (W m ⁻²) * Precipitation corrected (mm day ⁻¹)	0.01	0.0002	43.31	0.0001	1.01	1.01	1.01

Note: Possible interactions between the variables are indicated by an asterisk. Significant *p* values are bolded and 0.0001 represents values that are extremely small to report. Confidence intervals and ratio of odds are also included where variables with bolded ratio of odds increased the likelihood of tetraploid presence as they increased.

TABLE 5 Soil nutrient (nitrogen, phosphorus, and potassium) and pH results of samples that were collected from four natural populations (three diploid [2x], one tetraploid [4x]) of *Rhodohypoxis baurii* var. *platypetala* that occur across the KwaZulu-Natal (KZN) part of the Drakensberg grasslands, South Africa.

Population ID	Population locality	Altitude (m a.s.l.)	Ploidy	Nitrogen (%)	Phosphorus (mg kg ⁻¹)	Potassium (mg kg ⁻¹)	pH
2	Highmoor Nature Reserve, KZN	1940	2x	0.49	41	104	4.1
6	Mt Gilboa Nature Reserve, KZN	1750	4x	1.35	89	247	4.3
13	Underberg, KZN	1530	2x	0.48	34	221	4.1
14	Underberg, KZN	1490	2x	0.20	25	98	4.1

Note: Altitudes (m a.s.l.) are shown for each population. Values presented are averages of the two samples taken per site. Population IDs correspond to the IDs in Table 1 and Figure 2.

DISCUSSION

In this study, we identified the ploidy level of 350 individuals from 17 populations of *R. baurii* var. *platypetala* across the KwaZulu-Natal and Free State provinces, South Africa, and then compared the environmental variables that characterized the sampled populations to test for possible relationships with ploidy level distributions. We found evidence for diploid, triploid, and tetraploid individuals. We compared leaf and flower traits between diploids and tetraploids and found that tetraploids possessed broader leaves and larger flowers than diploids and were generally larger than diploids, representing the gigas effect. The sampled populations were largely represented by a single ploidy (either diploid or tetraploid), except for one population that comprised both diploid ($n=27$) and triploid ($n=7$) individuals. We found that diploid and tetraploid individuals did not co-occur, which suggests that there is some geographical separation between these two ploidy levels. Comparisons of environmental variables suggest this separation is likely due to local-scale niche differentiation, specifically differences in soil composition and climatic conditions between diploid and tetraploid populations.

Our ploidy level distribution findings are not consistent with general predictions that polyploids are more prevalent in high altitudes and latitudes than diploids (see Husband et al., 2013 for review). Instead, we found that altitude did not significantly differ between diploid and tetraploid populations. While there was some overlap in the altitudinal range of diploid and tetraploid populations, we found that tetraploids did not occur at altitudes above 1750 m a.s.l., while diploids reached 2060 m a.s.l. Our pattern is consistent with other work from the northern hemisphere where diploids occurred at higher altitudes than polyploids (e.g., Afonso et al., 2021; Hardy et al., 2000). In the mixed-ploidy grass *Festuca amethystina* (Poaceae), diploids occurred at higher altitudes than tetraploids and they were not found at altitudes below 500 m a.s.l., while tetraploids did not occur at altitudes above 2000 m a.s.l. throughout Central and South-Eastern Europe (Kiedrzyński et al., 2021). In the Eastern Alps, *Senecio carniolicus* (Asteraceae) hexaploids occurred below 2500 m a.s.l., while diploids occurred above 2500 m a.s.l. (Schönswetter et al., 2007). While comparable with our findings, it is possible that for many plant taxa found in the northern hemisphere, their ploidy level distribution pattern may be attributable to the range expansion of polyploids in lower-altitude areas from the Pleistocene refugia (e.g., *F. amethystina*; Kiedrzyński et al., 2021), but this scenario does not explain why tetraploids of *R. baurii* var. *platypetala* occur at lower altitudes than diploids in the Drakensberg grasslands. Moreover, a similar belowground investment was observed between the diploids and tetraploids (M. P. Mtileni, K. L. Glennon; unpublished data), so we do not have evidence to support the hypothesis that there may be a differential ploidy-level morphological response to fire in the Drakensberg.

We found that in addition to occurring at lower altitudes than diploids, tetraploids generally occurred in warmer conditions, in drier soils, and experienced increased infrared radiation intensity (more heat) than did diploids, suggesting that the tetraploids occur in more stressful environments at generally lower altitudes than their diploid progenitors. Although tetraploid populations received more rain, on average, than diploid populations, the soils may be more likely to dry out more quickly due to the generally higher temperatures and increased infrared radiation intensity that cause water to rapidly evaporate, possibly reducing soil water availability for tetraploids. Recent work on this taxon did not find significant differences in ploidy level responses to water availability (Mtileni et al., 2021). The difference in temperature conditions between the diploid and tetraploid populations

makes sense because of the fine-scale variations in near-surface temperature across the Drakensberg between footslope and scarp edge locations (Grab, 2013). Tetraploids also occurred in more humid conditions than diploids which may be due to increased precipitation, higher temperatures and increased infrared radiation intensity, and subsequent evaporation in tetraploid populations that can increase humidity.

While daily data of all 13 climatic variables differed significantly between the diploid and tetraploid populations and overall, the variables' means were higher for tetraploid populations compared to diploid populations, we acknowledge the possibility that these differences are descriptive and likely population-specific, so they do not necessarily predict the presence of tetraploids. For instance, while tetraploid populations were characterized by high mean temperature, precipitation, and infrared radiation intensity and low soil moisture, there was some overlap between diploid and tetraploid populations regarding these variables. We found that of all the variables considered in this study, only increased infrared radiation intensity and an interaction of increased infrared radiation intensity and precipitation increased the likelihood of tetraploid presence as they increased, while the other variables reduced this likelihood as they increased.

Diploids and polyploids occupying different climatic habitats have also been observed in both the southern and northern hemispheres. In *C. angustifolium* (Onagraceae), a similar pattern to our findings was found where pure tetraploid populations occurred in warmer and drier habitats than pure diploid populations across North America, Greenland, and Iceland (Thompson et al., 2014). In the mountain ranges of central Argentina, diploids of *Turnea sidoides* subsp. *pinnatifida* (Turneraceae) grew at higher altitudes in colder and more humid conditions, while hexaploids occurred in drier and warmer conditions at lower altitudes (Elías et al., 2011). In the Eastern Alps, tetraploid populations of *Cardamine amara* (Brassicaceae) were found at higher altitudes, in colder conditions with more intense solar radiation and higher precipitation, than diploid populations (Zozomová-Lihová et al., 2015). These findings, together with ours, indicate that local-scale temperature and soil properties are key determinants of polyploid distribution patterns, particularly in mountain environments.

Here, we found that both ploidy levels occurred in acidic soils, regardless of altitude and locality, but a trend was observed where a tetraploid population occurred in a habitat with marginally higher nitrogen and phosphorus availability than three diploid populations. The distribution of polyploid individuals could be restricted in soil with low availability of nitrogen and phosphorus due to greater demands of these nutrients by the nucleus as duplicated chromosome sets are costly to build and maintain (Faizullah et al., 2021; Leitch & Leitch, 2008). We acknowledge that further research is needed to compare soil nutrients between tetraploids and diploid populations. According to our present results, it is possible that while tetraploids may benefit from tolerance to increased temperatures and drier environments than diploids, their establishment and abundance could be restricted in nutrient-poor conditions where they can be outcompeted for resource acquisition by diploids. The increased performance of polyploids in more nutrient-rich conditions (e.g., more available nitrogen and phosphorus), and the potential establishment in such environments compared to diploids, points to their increased demand for nitrogen and phosphorus than diploids (Anneberg & Segraves, 2020; Walczyk & Hersch-Green, 2019). In *Epilobium (Chamerion) angustifolium* ssp. *angustifolium* (Onagraceae), tetraploids accumulated more belowground and aboveground biomass and increased root bud production under high soil nitrogen and phosphorus conditions, whereas the

same conditions did not have a significant effect on diploids (Walczyk & Hersch-Green, 2019). Further, in *Arabidopsis thaliana* (Brassicaceae), growth and composite fitness were higher for tetraploids under increased nitrogen and phosphorus conditions compared to diploids (Anneberg & Seagraves, 2020). This means that the availability of soil nutrients may shape ploidy-level distributions in natural populations by hindering polyploid distribution in nutrient-poor environments. We hypothesize that the Drakensberg basalts, which contain lower available phosphorus concentrations compared to other common rocks (Porder & Ramachandran, 2013), may have selected against the tetraploids of *R. baurii* var. *platypetala* at high altitudes. Diploid and tetraploid populations that were sampled for this study occurred in the Drakensberg sandstone rock surfaces, which are found at lower altitudes than the basalts in this ecosystem. It is possible that any available soil nutrients are leached by rain from the diploid-occupied higher altitudes, which would then increase soil nutrients at lower altitudes, facilitating tetraploid distribution at lower altitudes than diploids.

Polyploidy can increase cell size and subsequently alter morphological and physiological traits across angiosperm plant species (Otto & Whitton, 2000). Here, we found that tetraploids possessed broader leaves and larger flowers than diploids. We also found that flower size was significantly correlated with increased infrared radiation intensity (more heat) and precipitation, suggesting that both ploidy and environmental variables may explain the morphological differentiation between diploids and tetraploids. Moreover, it is possible that tetraploids are able to establish faster than diploids in habitats with increased nitrogen and phosphorus because these nutrients are readily available to maintain their larger plants (proxy for increased cell size). More research to assess different trait responses of these plants to both nutrient-limited and nutrient-rich conditions is necessary.

CONCLUSION

We show that sampled diploid and tetraploid individuals of *R. baurii* var. *platypetala* occur in different environmental conditions that might explain a nonrandom geographical separation of the ploidy levels across the Drakensberg grasslands. Reciprocal transplant experiments that investigate the potential establishment of ploidy levels in the habitat of the other ploidy level, and measurements of potential trait plasticity in such environments, will improve our understanding of the general distribution of polyploid plants at local scales. Our work shows that the geographic distribution of *R. baurii* var. *platypetala* ploidy levels is likely driven by responses to soil factors and temperature.

AUTHOR CONTRIBUTIONS

Masingitla P. Mtileni: Conceptualization (supporting); data curation (lead); formal analysis (lead); writing – original draft (lead); writing – review and editing (supporting). **Kenneth C. Oberlander:** Resources (supporting); software (supporting); supervision (supporting); writing – review and editing (supporting). **Kelsey L. Glennon:** Conceptualization (lead); funding acquisition (lead); resources (lead); supervision (lead); writing – original draft (supporting); writing – review and editing (supporting).

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

Flow cytometric fcs files for ploidy levels are available at https://osf.io/e8k2g/?view_only=566e3f2f38194a42ae0c33f8c6e39559.

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SUPPORTING INFORMATION

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