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2024-09

University of the Witwatersrand, Johannesburg

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Mtileni, Masingitla Promise. (2024). Examining intraspecific ploidy variation and functional traits that facilitate the environmental distribution of the Drakensberg near-endemic species, Rhodohypoxis baurii (Baker) Nel. var. platypetala Hilliard & Burtt. [PhD thesis, University of the Witwatersrand, Johannesburg]. WIReDSpace. https://hdl.handle.net/10539/46907

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EXAMINING INTRASPECIFIC PLOIDY VARIATION AND FUNCTIONAL TRAITS THAT FACILITATE THE ENVIRONMENTAL DISTRIBUTION OF THE DRAKENSBERG NEAR-ENDEMIC SPECIES, *RHODOHYPOXIS BAURII* (BAKER) NEL. VAR. *PLATYPETALA* HILLIARD & BURTT

by MASINGITLA PROMISE MTILENI

A Thesis submitted to The Faculty of Science of the
University of the Witwatersrand, Johannesburg,
in fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Thesis supervised by Kelsey L. Glennon
Associate Professor of Evolutionary Biology

SEPTEMBER 2024

DECLARATION

I declare that this thesis is my own unaided work except where I have explicitly indicated otherwise. It has not been submitted before for any degree or examination at any other University or similar institution and does not contain material previously published by another person except where I have explicitly indicated otherwise.



Masingitla P. Mtileni

September 12, 2024

ABSTRACT

Polyploidy is widely acknowledged as a key feature of evolution in flowering plants. Polyploidy often leads to reproductive isolation between polyploids and their diploid progenitors. This can arise via morphological and habitat differentiation, with polyploid plants often exhibiting increased growth, survival, and reproductive output under different environmental conditions. However, whether polyploidy drives general ecological divergence between diploids and polyploids or confers an advantage for plants under different environmental conditions remains an open question. Polyploids are generally thought to have an increased adaptive potential to populate areas that are increasingly environmentally 'extreme'. These 'extremes' can be described as experiencing the extreme end of an environmental variables; either colder or hotter temperatures, drier or wetter habitats, increased UV exposure, or increased/decreased seasonality. The ability of polyploids to acclimate to these 'extremes' may be a consequence of the beneficial outcomes of polyploidy, such as intrinsically higher heterozygosity and altered gene expression.

My thesis examines how environmental variables may shape the distribution of plants with different ploidies, the association of polyploidy with plant morphotypes, and whether plants with different ploidy levels respond distinctively to different environmental conditions. Using a meta-analysis, I examined the hypothesis that there is consistent segregation between diploid and polyploid plants based on elevation. I found that the perceived trend of polyploids occurring at significantly higher elevations than diploids was not supported for 71% of the sampled 28 studies. In addition, climate variables, soil moisture, and geographic region only explained 0.3% of polyploid presence at the highest elevations across studies. Next, I conducted a cytogeographic study, growth chamber and greenhouse experiments, and reciprocal transplants to examine ploidy level distribution and the association of polyploidy with the responses of plants to different environmental conditions at the population level. I

focused on *Rhodohypoxis baurii* var. *platypetala* (Hypoxidaceae), a Drakensberg near-endemic taxon. I found evidence for both habitat and morphological differentiation between diploid and tetraploid populations, where tetraploids had larger leaves and larger flowers and generally occurred at lower elevations in warmer and drier habitats with soil that is marginally higher in nitrogen and phosphorus concentration than diploid habitats. Only one mixed-ploidy population (diploids and triploids) was found. My findings suggest that a higher ploidy level was not associated with larger morphological traits or increased reproductive output under greater soil nitrogen, phosphorus, and salinity levels, nor greater survivorship and increased reproductive output under higher salinity, or after being transplanted.

Together, findings from this thesis dovetail with collective findings from other work where increased ploidy was not found to confer an advantage or limitation under different environmental conditions, and where the general relationship between environmental variables and polyploid distribution was found to be indirect or negligible. Work at large scales (e.g., broad scale and at a global level) may reveal meaningful polyploid patterns that may not be evident at smaller scales. However, such work may not be accurate when predicting the role of polyploidization in plants generally or outlining the general biogeographic principle of polyploid distribution due to the lacking historical component which prevents us from inferring nascent drivers of observed distributions. Broad comparative work should be complemented with robust experimental studies (e.g., reciprocal transplants and common garden experiments). Comparing niches of diploid and polyploid populations from the same species may also be more useful in identifying unifying trends than comparing niches of polyploid species across the globe.

ACKNOWLEDGEMENTS

I would like to greatly thank my advisor, Prof. Kelsey L. Glennon, for her stellar supervision and mentorship since 2017. Thank you for inspiring me to strive for excellence, consistency, and integrity during my postgraduate journey, and beyond my studies. Thank you for everything. I am also grateful to my family and friends for their unwavering support and for standing with me to the completion of this thesis. Special thanks to Zuko, Travar, Tomi, Pheello, with love and respect. Zuko, thank you for your overall company and support, and for the encouragement when I struggled to move ahead with the writing. Thando, Dineo, Evalt, Flavia, Jess, William, I thank you. Many thanks to Thato for being an amazing field assistant and for the genuine discussions centered around polyploidy. Many thanks to members of the PEAS Lab at the University of the Witwatersrand for their input and support. I would like to give special thanks to Hennie and Bianca for their assistance with field work, and I greatly thank Hennie for his assistance with growth chamber experiments, lab work, and flow cytometric analyses. Hennie, Saness, Verona, Annika, Sipho, Dewald, thank you for the emotional support. Dr. Stephanie L. Payne-Smith, thanks for your invaluable support and feedback. Dr. Safiyyah Iqbal, thank you for the support. My work on this thesis was supported by a Doctoral Scholarship (131342) from the National Research Foundation, South Africa. Many thanks to the University of the Witwatersrand for awarding me the Postgraduate PhD Merit Award. I thank the Almighty God for everything in my life.

With a full heart and love + respect, I humbly dedicate this work to my beloved mother, Rifumuni Maria Mtileni.

"We will have eternity to celebrate the victories but only a few hours before sunset to win them." **– Amy Carmichael**

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Published in *Austral Ecology* as:
Mtileni MP, Oberlander KC, and Glennon KL. 2024. Morphological and habitat differentiation between diploids and tetraploids of a Drakensberg near-endemic taxon, *Rhodohypoxis baurii* var. *platypetala* (Hypoxidaceae). *Austral Ecology* 49: e13502. https://doi.org/10.1111/aec.13502
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CHAPTER 1

GENERAL INTRODUCTION



Flowering *Rhodohypoxis baurii* var. *platypetala* plants in a wild population in the KwaZulu-Natal province, South Africa.

Rationale

Polyploidy, a condition of possessing more than two sets of chromosomes through intraspecific whole genome duplication (autopolyploidy, homologous genomes) or interspecific hybridization (allopolyploidy, nonhomologous genomes), is considered a key evolutionary force for plant species and populations. Abundant research has associated polyploid success with environmental change or 'stress' due to mechanisms, such as increased genetic variation, changes in gene expression, and increased adaptive potential in polyploid plants (Ramsey and Ramsey, 1998; Husband *et al*., 2013; Van de Peer *et al*., 2017, 2021). For example, polyploid plants may exhibit an increased stress tolerance and survival under water stress and salinity relative to diploid plants (Maherali *et al*., 2009; Zhang *et al*., 2010; Liu *et al*., 2011; Chao *et al*., 2013; Yang *et al*., 2014). Polyploids may also survive, grow, and reproduce better than diploids in nutrient enriched environments because of the higher requirements of nitrogen and phosphorus to build larger genomes (Leitch and Leitch, 2008; Guignard *et al*., 2016; Walczyk and Hersch-Green, 2019; Annerberg and Segraves, 2020; Faizullah *et al*., 2021). However, whether polyploidization drives general ecological divergence and differential ploidy-level responses under different environmental conditions remains an open question. Polyploidization may not always lead to ecological divergence between diploids and polyploids (Glennon *et al*., 2014; but see Thompson *et al*., 2014; Visger *et al*., 2016), and there may not always be a direct relationship between polyploid plants and how they respond to environmental change or 'stress' (Wei *et al*., 2019, 2020; Mtileni *et al*., 2021; Harms *et al*., 2021). One common hypothesized avenue for polyploid plants is that they are better suited to establish in 'extreme/stressful' environments than diploids, as demonstrated by the occurrence of polyploid plant species across latitudes and certain biomes (Rice *et al*., 2019). For instance, polyploid plants are thought to generally occur in higher

latitudes and higher elevations than diploids. Higher latitudes and higher elevations are often characterized by extreme cold conditions, extreme temperature fluctuations, and highly variable moisture availability (Husband *et al*., 2013; Bureš *et al*., 2024). For example, polyploid frequency increases northwards into the arctic (Brochmann *et al*., 2004). Polyploidization has also been associated with 'extreme' periods in geological history, such as Cretaceous-Paleogene boundary (Lohaus and Van de Peer, 2016) and with high resistance to low freezing temperatures, elevated ultraviolet, and drought (Bomblies, 2020). Several factors have been suggested to explain polyploid prominence in these 'extreme' environments, including increased genetic variation and increased adaptive potential (Ramsey and Ramsey, 1998; Comai, 2005; Husband *et al*., 2013; Van de Peer *et al*., 2017, 2021). Cold and hot temperatures have also been linked with the formation of unreduced gametes (Ramsey and Ramsey, 1998; Van de Peer *et al*., 2017; He and Lamont, 2018; Glennon *et al*., 2024), which could bolster the generation of polyploids in these 'extreme' spaces.

While the trend of polyploids populating relatively extreme cold conditions at higher latitudes is a striking pattern and has been consistent at a global scale (Rice *et al*., 2019; Bureš *et al*., 2024), it is not consistent at local scales where polyploids have been found to occur at higher elevations and higher latitudes than diploids (e.g., Krejčíková *et al*., 2013; Visger *et al*., 2016), but also at lower elevations and lower latitudes than diploids in different species (e.g., Kiedrzyński *et al*., 2021; Chumová *et al*., 2023; see Table 1.1 for 16 examples of studies where diploids occurred at higher latitudes and/or higher elevations than polyploids). Collectively, these findings suggest that the ecological consequences experienced by polyploids may differ across species and populations. For instance, in the Alps, *Cardamine amara* L. (Brassicaceae) polyploids tend to occur in colder, higher radiation, and higher rainfall conditions than diploids (Zozomová-Lihová *et al*., 2015)

whereas in the mountain ranges of central Argentina, *Turnea sidoides* subsp. *pinnatifida* (Juss. Ex Poir) Arbo (Turneraceae) diploids occur in colder conditions (Elías *et al*., 2011). More research at local scales may help refine our understanding of general patterns of polyploid distribution and the putative role of polyploidy in plant evolution and biodiversity.

Recently, the Northern Hemisphere was found to have a higher polyploid frequency compared to the Southern Hemisphere (Rice *et al*., 2019; Bureš *et al*., 2024). This finding demonstrates the need to examine the presence or absence of polyploidy in the Southern Hemisphere where polyploidy has been largely underexplored. For instance, the Greater Cape Floristic Region has some evidence of polyploidy (Krejčíková *et al*., 2013; Oberlander *et al*., 2016; Linder *et al*., 2017; Andrés-Sánchez *et al*., 2019; Veselý *et al*., 2020; Dogan *et al*., 2021; Elliott *et al*., 2023; Chumová *et al*., 2023), where both genome size and ploidy levels have been associated with the traits and environmental distribution of different plant taxa. Here, I evaluated the influence of the environment on polyploid plant distribution, how polyploidy may shape plant morphotypes, and whether ploidy level may affect plant traits and growth in different environmental conditions. I focused on a plant taxon that is a near-endemic to the Drakensberg region, South Africa, which is a biodiversity hotspot (Carbutt and Edwards, 2003, 2006). One of the reasons behind the biodiversity in this ecosystem may be the presence of polyploids. To address my questions, I focused on *Rhodohypoxis* Nel. (Asparagales: Hypoxidaceae), a small Drakensberg near-endemic plant genus that exists mainly in the mountain grasslands of the KwaZulu-Natal Drakensberg in South Africa (see details below; Hilliard and Burtt, 1978).

Polyploidy and plant evolution

Abundant work highlights polyploidy as a prevalent and key evolutionary force for plant species and populations. Polyploidy is an important driver of adaptation and speciation

in flowering plants (Sobel *et al*., 2010; te Beest *et al*., 2012; Husband *et al*., 2013; Ramsey and Ramsey, 2014). This condition is common across plant lineages, across the Southern Hemisphere and the Northern Hemisphere, and across different biomes (Rice *et al*., 2019; Bureš *et al*., 2024). Stebbins (1938) estimated that up to 40% of angiosperms were polyploid, and later on, Masterson (1994) suggested that approximately 70% of angiosperms experienced polyploidization. Soltis and Soltis (1999) emphasized that polyploidy can form recurrently in different populations of their diploid progenitors and not only through a single origin as previously thought, with potential for increased genetic variation due to hybridization across different parental genotypes. Polyploidy is thought to be responsible for the genome size diversity seen across plant taxa, ranging ca. 2400-fold (Pellicer *et al*., 2018). Abundant work showed a positive relationship between genome size associated with higher ploidy levels and cell size where polyploids generally possess larger stomatal guard cells than diploids (i.e., larger but fewer stomata; Masterson, 1994; Beaulieu *et al*., 2008; Liu *et al*., 2011; Wei *et al*., 2019, 2020; Domínguez-Delgado *et al*., 2021; van Mazijk *et al*., 2024). Recently, larger genome sizes were associated with larger but fewer stomata and potentially higher water use efficiency in sedges in the Cape region of South Africa (van Mazijk *et al*., 2024). Other polyploid outcomes in plants include morphological differentiation (e.g., larger leaves and flowers in polyploids compared to diploids; Yu *et al*., 2010; Liu *et al*., 2011; Porturas *et al*., 2019) and climatic niche differences between diploids and polyploids (Thompson *et al*., 2014; Visger *et al*., 2016; but see Glennon *et al*., 2014; Marchant *et al*., 2016). Prezygotic (e.g., increased self-fertilization or asexual reproduction in polyploids, or ecogeographic isolation between ploidy levels) and postzygotic (e.g., 'triploid block') reproductive barriers have also been found between polyploids and their progenitors (Barringer, 2007; Glennon *et al*., 2012), potentially reducing gene flow between ploidy levels

and facilitating speciation (Husband and Sabara, 2004; Yu *et al*., 2010; Hersch-Green, 2012; but see Barringer and Galloway, 2017).

Detecting polyploids

Confirming polyploid presence is important for foundational work regarding the significant role of polyploid formation and its putative implications for plant ecology and evolution. Methods, such as chromosomal squashes, have been used for determining chromosome number or ploidy levels in plants (i.e., ploidy level estimates based on different chromosome numbers), but flow cytometry is a commonly used technique to measure nuclear DNA content in plant cells because it is cost-effective, flexible, and produces rapid, accurate and reproducible results, although samples with known chromosome numbers are needed for calibration against the inferred ploidy levels (Doležel *et al*., 2007; Loureiro *et al*., 2023). Flow cytometry uses DNA-selective fluorochromes, like DAPI (4,6-diamidino-2-phenylindole) which binds to the adenine-thymine-rich sequences in DNA, to stain particles (e.g., cells or nuclei) of a sample and an appropriate standard; these stained particles are compared by relative fluorescence intensities that correspond to DNA content, which are used to infer ploidy level or genome size (Doležel *et al*., 2007). DAPI has emerged as a commonly used and widely applicable stain due to its provision of high-resolution histograms of DNA content relative to stains such as propidium iodide (Doležel *et al*., 2007). The extensive use of flow cytometry on different plant systems demonstrates the impact of this technique for providing invaluable findings regarding plant ecology and evolution based on ploidy level and genome size (Galbraith *et al*., 2021). Data are readily available on various databases where flow cytometry has been recommended as best practice and used to generate DNA contents for thousands of plant systems (e.g., https://cvalues.science.kew.org/; Pellicer

and Leitch, 2020), further demonstrating the utility and impact of the technique in boosting plant biology research.

Polyploid establishment and distribution

Polyploids form repeatedly within plant populations (Soltis and Soltis, 1999) and unreduced gametes primarily contribute to the formation of polyploid plants (Ramsey and Schemske, 1998). Unreduced gametes (UG) are maintained by genetic factors (e.g., mutation-selection balance, Ramsey and Schemske, 1998; Kreiner *et al*., 2017) and UG production may vary among species in natural populations (Kreiner *et al*., 2017), but in some instances, UG can be induced by environmental factors such as temperature (Pécrix *et al*., 2011). Two main types of polyploidy are recognized based on mode of origin, although gains and losses of single chromosomes due to aneuploidy (duplication or loss of chromosomes implying change in DNA content) and dysploidy (fusion and/or fission of chromosomes implying no change in DNA content) are also possible: autopolyploidy, which involves intraspecific whole genome duplication, and allopolyploidy, which involves the hybridization of different genomes (for review, see Ramsey and Schemske, 1998), although there is also a recognized continuum between the two. Autopolyploids are formed by gametic nonreduction or somatic doubling within a diploid population or through triploids that self-fertilize or backcross to diploids to produce tetraploids (Ramsey and Schemske, 1998). Allopolyploids can be formed through interspecific crosses between diploids, a hybridization event between distinct autopolyploids, or triploids that self-fertilize or backcross to diploids to produce tetraploids (Ramsey and Schemske, 1998).

Beneficial outcomes of polyploidization in plants include increased heterozygosity and genetic diversity, changes in gene expression, masking of deleterious alleles, redundancy in gene functions, and hybrid vigour and subsequent competitiveness, all of which may

enhance the ability of polyploid plants to respond to a wide range of environmental conditions (for review, see te Beest *et al*., 2012). For instance, an increase in ploidy may enable a fitness advantage for polyploid plants exposed to higher or lower levels of salinity (Chao *et al*., 2013). Plants with more DNA may need to occupy areas with increased nitrogen and phosphorus as larger genomes require these nutrients to build and maintain the increased amounts of DNA (Guignard *et al*., 2016; Walczyk and Hersch-Green, 2019; Annerberg and Segraves, 2020; but see Harms *et al*., 2021), and confer adaptation to novel environments (Ramsey, 2011). The disadvantages of polyploidy include difficulties in mitosis and meiosis which may lead to the loss of chromosomes and the formation of aneuploid cells that can trigger genomic and epigenetic instability (Comai, 2005). Polyploidy may also affect and reduce fertility and fitness in plants due to difficulties associated with the completion of cell division (te Beest *et al*., 2012).

Polyploid lineages can be found across the globe (Rice *et al*., 2019; Bureš *et al*., 2024) but first, polyploid individuals must survive and successfully establish in their local populations. Because polyploids are initially rare in a population, post-zygotic reproductive isolation between individuals of different ploidy levels may lead to minority cytotype exclusion (MCE) where polyploids may go extinct (Levin, 1975). Local-scale work focusing on environmental factors that are associated with successful polyploid establishment (polyploids exist) is necessary as polyploid outcomes may differ across species and populations. In the absence of habitat differentiation between diploids and polyploids, the association between high rates of self-fertilization and polyploidy can mitigate MCE (Barringer, 2007). Minority cytotype exclusion can also be weakened by polyploid plants occupying larger range sizes to take advantage of disturbed or vacant habitats where species competition is potentially low (e.g., Kiedrzyński *et al*., 2021). Ploidy level distribution has also been associated with life history traits where polyploid abundance increases with a high

frequency of perennial herbs, allowing polyploid plants time and space to compete with their progenitors (Rice *et al*., 2019).

Studies at large scales (e.g., across biomes or global measures) focusing on polyploid distribution patterns are necessary for understanding polyploid plant ecology and evolution as these studies reveal emergent patterns that are not evident at small scales. However, large-scale work may be limited in predicting ploidy level distribution patterns at small scales (e.g., within localities) due to broad generalizations for different species and populations where taxonomic bias or uneven sampling efforts across taxa and geographic regions may not be accounted for. The coarse-resolution in large-scale studies may conceal or overlook effects of environmental variables that have been shown to be important for polyploid establishment in different species. For instance, treatments or areas with elevated nitrogen and phosphorus levels have been shown to favour the growth of polyploid plants due to the higher requirements of these nutrients after a genome size increase (e.g., Guignard *et al*., 2016; Walczyk and Hersch-Green, 2019; Annerberg and Segraves, 2020), yet phosphorus availability in the soil was found to have a low explanatory power on global polyploid distribution (Rice *et al*., 2019). Comparing diploid and polyploid populations from the same species is also more useful than only looking at polyploid species across the globe (e.g., Parisod and Broennimann, 2016; Marchant *et al*., 2016).

Drakensberg climate and soil heterogeneity

The Greater Drakensberg Escapement, located in the grassland biome in southern Africa and including the plateau across South Africa and Lesotho, is known for comprising high angiosperm-species richness, although some families (e.g., Asteraceae and Fabaceae) are more common than others (e.g., Hypoxidaceae and Brassicaceae; Carbutt and Edwards, 2003, 2006). The Drakensberg region also hosts endemics and near-endemics (Carbutt and

Edwards, 2003, 2006). The floristic diversity in this mountain region may be attributed to climate and soil heterogeneity that changes dramatically as one climbs in elevation (peaks reach over 3000 m a.s.l.). Environmental heterogeneity in soil, temperature, and precipitation are found at small spatial scales (< 10 km; Grab, 2013; Knight and Grab, 2015). For instance, using hourly data of air temperature recorded over a year in this region, Grab (2013) found evidence for increasing temperature lapse rates from –0.42 100 m^{-1} to –0.84 °C 100 m^{-1} with an elevation gain of 550 m a.s.l. Previous work in this region also found high and consistent mean annual rainfall ranging 750–1350 mm with more summer rainfall (Nel, 2008), and high frost cycle frequency in the air and at the soil surface for the winter season (Nel and Sumner, 2008). In addition to climate, two distinct rock types occupy different spatial scales, with basalts capping the Drakensberg and sandstones are found predominantly below 2000 m a.s.l. (Knight and Grab, 2015). The Drakensberg is characterized by low pH soils where nutrients are potentially leached out to lower elevations due to snow melt, rainfall, and steep slopes (Carbutt and Edwards, 2015).

Polyploidization may have contributed to the biodiversity in the Drakensberg, but this ecosystem is underexplored regarding polyploid work compared to the geographically close but floristically dissimilar Greater Cape Floristic Region. In the Greater Floristic Region, there is some work on the association between polyploidy and plant traits and potential differential responses of plants with different ploidies to different environmental conditions, and the influence of environmental conditions on ploidy level distribution (e.g., Krejčíková *et al*., 2013; Veselý *et al*., 2020; Elliott *et al*., 2023, Chumová *et al*., 2023). There is potential for a growing, focused body of work on polyploid biology, plant ecology, and evolution in this ecosystem.

Polyploidy in Hypoxidaceae

Rhodohypoxis Nel. (Asparagales: Hypoxidaceae) is a small plant genus comprising six species of herbaceous perennial geophytes that exist in the mountain grasslands of the KwaZulu-Natal Drakensberg in South Africa, Lesotho, and into Eswatini (Hilliard and Burtt, 1978). The genus-level taxonomy is contentious, so this thesis follows the current taxonomic designation by Hilliard and Burtt (1978). This thesis focuses on the most widespread species of this genus, *R. baurii* (Baker) Nel, from which Hilliard and Burtt (1978) described three varieties ('taxa' hereafter) using morphological traits and ecological distribution. In summary, *R. baurii* var. *confecta* grows on damp grass slopes and produces erect and narrow bright green leaves and sheets of mixed white, pink, or bright pink flowers; *R. baurii* var. *baurii* grows in seasonally moist habitats and produces long, erect and narrow green leaves and deep red or pink flowers; *R. baurii* var. *platypetala*, the focal taxon of this thesis, grows in dry ground, often in shallow stony soils over rocky sheets and produces grey-green, broad and flat leaves and predominately white flowers which are occasionally pale pink in the centre or outside, or greenish on the back (Fig. 1.1, 1.2). This taxon is the most widespread and morphologically largest variety of *R. baurii*, and it occurs in localities that comprise an elevation gradient that is useful to test the effect of environmental patterns of different ploidies in a heterogeneous mountain environment. Saito (1975) showed chromosomal squash evidence for the presence of diploids ($2n = 12$) and triploids ($2n = 18$) in *R. baurii* var. *platypetala* from individuals that were collected from the Eastern Cape province, South Africa, but a comprehensive study to examine the extent of the intraspecific ploidy variation in wild populations was not undertaken. *Rhodohypoxis baurii* var. *platypetala* is a useful system to improve our understanding of how the environment may dictate where different ploidy levels can grow, how polyploidy affects plant trait variation, and potential responses by plants with different ploidy levels to different environmental conditions that may be experienced in a heterogeneous mountain environment. Polyploidy has also been associated

with clonal taxa (e.g., *Schoenus*, van Mazijk *et al*., 2024), such as *R. baurii* var. *platypetala*, which raises questions about the role of intraspecific ploidy variation on plant traits and environmental distribution in this taxon. There is an ongoing interest in how polyploidy likely contributes to the evolution of this group in the Drakensberg. Of the six endemic Drakensberg taxa, this genus is the most straightforward to work with in terms of feasibility, life history, and ease of cultivation, and given the paucity of work in the Drakensberg, focusing on a near-endemic genus is helpful to understand what factors might drive polyploidy in this region. Work on this genus highlighted that polyploidy affects leaf traits (e.g., larger stomata in tetraploids compared to diploids), but not water use efficiency under water stress (Mtileni *et al*., 2021). Previous work also evaluated population genetics in this group (Ferreira *et al*., in prep), so there is a general background knowledge of the biology and evolutionary history of the group.

Thesis Aim and Objectives

Work within this thesis investigates how environmental variables shape the distribution of different ploidy levels of plant species and populations at a local and then at a global scale. I used three objectives to address this aim:

Objective 1: Conduct a meta-analysis to synthesize the effect size direction and magnitude of the distribution of diploid and polyploid populations based on elevation and its correlated variables using studies of different plant systems and geographic regions. Here, I first examined whether increasing elevation and associated environmental variables (climate and soil) are associated with broad-scale (within regions) polyploid distribution. To specifically focus on the focal variable (elevation), I then compared environmental variables at the highest diploid and highest polyploid elevations across these studies, and tested if any

environmental variables predicted the presence of polyploids at high elevations across studies.

Objective 2: Investigate the intraspecific ploidy variation in *R. baurii* var. *platypetala* across its range to assess geographic or environmental separation of different ploidies. This objective comprises a cytogeographic study to map out the localities and associated habitats of different ploidy levels.

Objective 3: Evaluate how environmental variables (e.g., climate and soil) influence plant traits in different locations. Here, I compared morphological traits of different ploidies across different populations, and used a growth chamber experiment to assess and compare the vegetative and floral response, reproductive output, and survivorship of diploid and polyploid individuals under soil nitrogen, phosphorus, and salinity treatments. I then used a reciprocal transplant experiment to assess and compare the survivorship, establishment, potential phenotypic trait response, and reproductive output of diploid and polyploid individuals after they were reciprocally transplanted between two Drakensberg sites.

Thesis Structure

The objectives above are written as three data chapters. This thesis comprises a general introduction, three data chapters, and a general conclusion. Each data chapter is written as a separate manuscript, so repetitions of information between them may be noted, specifically in the introduction and materials and methods. The general conclusion provides a synthesis of the thesis' main findings in a broader context of the relationship between ploidy and plant environmental distribution, traits and potential response to different environmental conditions, and concluding remarks. The data chapters were formatted for their respective journals, so formatting may differ between the chapters. Chapter 3 has been published in

Austral Ecology. Chapter 2 has been prepared for submission to *New Phytologist*, and chapter 4 has been prepared for submission to *Journal of Experimental Botany*.

Literature Cited

Andrés-Sánchez S, Verboom GA, Galbany-Casals M, *et al*. 2019. Evolutionary history of the arid climate-adapted *Helichrysum* (Asteraceae: Gnaphalieae): Cape origin and association between annual life-history and low chromosome numbers. *Journal of Systematics and Evolution* 57:468–487

Anneberg TJ, Segraves KA. 2020. Nutrient enrichment and neopolyploidy interact to increase lifetime fitness of *Arabidopsis thaliana*. *Plant and Soil* 456:439–453

Afonso A, Loureiro J, Arroyo J, *et al*. 2021. Cytogenetic diversity in the polyploid complex *Linum suffruticosum* s.l. (Linaceae). *Botanical Journal of the Linnean Society* 195:216–232

Barringer BC. 2007. Polyploidy and self-fertilization in flowering plants. *American Journal of Botany* 94:1527–1533

Barringer BC, Galloway LF. 2017. The reproductive ecology of diploid and tetraploid *Galax urceolata. The American Midland Naturalist* 177:299–308

Beaulieu JM, Leitch IJ, Patel S, *et al*. 2008. Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytologist* 179:975–986

Bomblies K. 2020. When everything changes at once: finding a new normal after genome duplication. *Proceedings of the Royal Society B* 287:20202154

Brochmann C, Brysting AK, Alsos IG, *et al*. 2004. Polyploidy in arctic plants. *Biological Journal of the Linnean Society* 82:521–536

Bureš P, Elliott TL, Veselý P, *et al*. 2024. The global biogeography of angiosperm genome size is shaped by climate and range size. *New Phytologist* 242:744–759

Carbutt C, Edwards TJ. 2003. The flora of the Drakensberg Alpine Centre. *Edinburgh Journal of Botany* 60:581–607

Carbutt C, Edwards TJ. 2006. The endemic and near-endemic angiosperms of the Drakensberg Alpine Centre. *South African Journal of Botany* 72:105–132

Carbutt C, Edwards TJ. 2015. Plant-soil interactions in lower-upper montane systems and their implications in a warming world: a case study from the Maloti-Drakensberg Park, southern Africa. *Biodiversity* 16:262–277

Chao DY, Dilkes B, Luo H, *et al*. 2013. Polyploids exhibit higher potassium uptake and salinity tolerance in *Arabidopsis*. *Science* 341:658–659

Chumová Z, Monier Z, Šemberova K, *et al*. 2023. Diploid and tetraploid cytotypes of the flagship Cape species *Dicerothamnus rhinocerotis* (Asteraceae): variation in distribution, ecological niche, morphology and genetics. *Annals of Botany* 133:851–870

Comai L. 2005. The advantages and disadvantages of being polyploid. *Nature Reviews Genetics* 6:836-846

Dogan M, Pouch M, Mandáková T, *et al*. 2021. Evolution of tandem repeats is mirroring post-polyploid cladogenesis in *Heliophila* (Brassicaceae). *Frontiers in Plant Science* 11: 607893

Doležel J, Greilhuber J, Suda J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols* 2:2233–2244

Domínguez-Delgado JJ, López-Jurado J, Mateos-Naranjo E, *et al*. 2021. Phenotypic diploidization in plant functional traits uncovered by synthetic neopolyploids in *Dianthus broteri*. *Journal of Experimental Botany* 72:5523–5533

Elías G, Sartor M, Neffa VGS. 2011. Patterns of cytotype variation of *Turnera sidoides* subsp. *pinnatifida* (Turneraceae) in mountain ranges of central Argentina. *Journal of Plant Research* 124:25–34

Elliott TL, Muasya AM, Bureš P. 2023. Complex patterns of ploidy in a holocentric plant clade (*Schoenus*, Cyperaceae) in the Cape biodiversity hotspot. *Annals of Botany* 131:143–156

Faizullah L, Morton JA, Hersch-Green EI, *et al.* 2021. Exploring environmental selection on genome size in angiosperms. *Trends in Plant Science* 26: 1039–1049

Galbraith D, Loureiro J, Antoniadi I, *et al*. 2021. Best practices in plant cytometry. *Cytometry Part A* 99:311–317

Girón V, Garnatje T, Vallès J, *et al.* 2012. Geographical distribution of diploid and tetraploid cytotypes of *Thymus* sect. *Mastichina* (Lamiaceae) in the Iberian Peninsula, genome size and evolutionary implications. *Folia Geobotanica* 47:441–460

Glennon KL, Rissler LJ, Church SA. 2012. Ecogeographic isolation: a reproductive barrier between species and between cytotypes in *Houstonia* (Rubiaceae). *Evolutionary Ecology* 26:909–926

Glennon KL, Ritchie ME, Segraves KA. 2014. Evidence for shared broad-scale climatic niches of diploid and polyploid plants. *Ecology Letters* 17:574–582

Glennon KL, Niemann HJ, Archibald S. 2024. Fire-mediated effects on polyploid biology. *Trends in Ecology & Evolution*. https://doi.org/10.1016/j.tree.2024.02.007

Grab SW. 2013. Fine-Scale variations of near-surface-temperature lapse rates in the high Drakensberg escarpment, South Africa: Environmental Implications. *Arctic, Antarctic, and Alpine Research* 45:500–514

Guignard MS, Nichols RA, Knell RJ, *et al*. 2016. Genome size and ploidy influence angiosperm species' biomass under nitrogen and phosphorus limitation. *New Phytologist* 210:1195–1206

Hardy OJ, Vanderhoeven S, De Loose M, *et al*. 2000. Ecological, morphological, and allozymic differentiation between diploid and tetraploid knapweeds (*Centaurea jacea*) from a contact zone in the Belgian Ardennes. *New Phytologist* 146:281–290

Harms NE, Cronin JT, Gaskin JF (2021) Increased ploidy of *Butomus umbellatus* in introduced populations is not associated with higher phenotypic plasticity to N and P. *AoB PLANTS* 13:plab045

He T, Lamont BB. 2018. Fire as a potent mutagenic agent among plants. *Critical Reviews in Plant Sciences* 37:1–14

Hersch-Green EI. 2012. Polyploidy in Indian paintbrush (*Castilleja*; Orobanchaceae) species shapes but does not prevent gene flow across species boundaries. *American Journal of Botany* 99:1680–1690

Hilliard OM, Burtt BT. 1978. Notes on some plants from southern Africa chiefly from Natal. VII. *Notes from the Royal Botanical Garden, Edinburgh* 36:43–76

Husband BC, Sabara HA. 2004. Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (Onagraceae). *New Phytologist* 161:703–713

Husband BC, Baldwin SJ, Suda J. 2013. The incidence of polyploidy in natural plant populations: major patterns and evolutionary processes. *Plant Genome Diversity Volume 2*, I.J. Leitch et al. (eds.), 255–276. Springer Vienna

Jalili A, Rabie M, Azarnivand H, *et al*. 2013: Distribution and ecological consequences of ploidy variation in *Artemisia sieberi* in Iran. *Acta Oecologica* 53:95–101

Kessler M, Kühn A, Solís Neffa VG, *et al*. 2014. Complex geographical distribution of ploidy levels in *Polylepis australis* (Rosaceae), an endemic tree line species in Argentina. *International Journal of Plant Sciences* 175:955–961

Kiedrzyński M. Zielińska KM, Jedrzejczyk I, *et al*. 2021. Tetraploids expanded beyond the mountain niche of their diploid ancestors in the mixed-ploidy grass *Festuca amethystina* L. *Scientific Reports* 11:18735

Knight J, Grab S. 2015. The Drakensberg Escarpment: mountain processes at the edge. S.W. Grab, J. Knight (Eds.), *Landscapes and Landforms of South Africa*, Springer Publishers, 47–55

Kreiner JM, Kron P, Husband BC. 2017. Frequency and maintenance of unreduced gametes in natural populations: associations with reproductive mode, life history and genome size. *New Phytologist* 214:879–889

Krejčíková J, Sudová R, Oberlander KC, *et al*. 2013. Cytogeography of *Oxalis pes-caprae* in its native range: where are the pentaploids? *Biological Invasions* 15:1189–1194

Levin DA. 1975. Minority cytotype exclusion in local plant populations. *Taxon* 24:35–43

Leitch AR, Leitch IJ. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* 320: 481–483

Linder HP, Suda J, Weiss-Schneeweiss H, *et al*. 2017. Patterns, causes and consequences of genome size variation in Restionaceae of the Cape flora. *Botanical Journal of the Linnean Society* 183:515–531

Liu X, Gituru WR, Wang Q. 2004. Distribution of basic diploid and polyploid species of *Isoetes* in East Asia. *Journal of Biogeography* 31:1239–1250

Liu S, Chen S, Chen Y, *et al*. 2011. In vitro induced tetraploid of *Dendranthema nankingense* (Nakai). Tzvel. shows an improved level of abiotic stress tolerance. *Scientia Horticulturae* 127:411–419

Lohaus R, Van de Peer Y. 2016. Of dups and dinos: evolution at the K/Pg boundary. *Current Opinion in Plant Biology* 30:62–69

Loureiro J, Čertner M, Lučanová M, *et al*. 2023. The use of flow cytometry for estimating genome sizes and DNA ploidy levels in plants. In *Plant Cytogenetics and Cytogenomics: Methods and Protocols*. New York, NY: Springer US, 25–64

Maherali H, Walden AE, Husband BC. 2009. Genome duplication and the evolution of physiological responses to water stress. *New Phytologist* 184:721–731

Masterson J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science* 264:421–424

Manzaneda AJ, Rey PJ, Bastida JM, *et al*. 2012. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *New Phytologist* 193:797–805

Marchant DB, Soltis DE, Soltis, PS. 2016. Patterns of abiotic niche shifts in allopolyploids relative to their progenitors. *New Phytologist* 212:708–718

Mtileni MP, Venter N, Glennon KL. 2021. Ploidy differences affect leaf functional traits, but not water stress responses in a mountain endemic plant population. *South African Journal of Botany* 138:76–83

Muñoz-Pajares AJ, Perfectti F, Loureiro J, *et al*. 2018. Niche differences may explain the geographic distribution of cytotypes in *Erysimum mediohispanicum*. *Plant Biology* 20:139–147

Nel W. 2008. Rainfall trends in the KwaZulu-Natal Drakensberg region of South Africa during the twentieth century. *International Journal of Climatology* 29:1634–1641

Nel W, Sumner P. 2008. Rainfall and temperature attributes on the Maloti-Drakensberg Escarpment Edge, southern Africa. *Geografiska Annaler* 90:97–108

Němečková H, Krak K, Chrtek J. 2018. Complex pattern of ploidal and genetic variation in *Seseli libanotis* (Apiaceae). *Annales Botanici Fennici* 56:57–77

Oberlander KC, Dreyer LL, Goldblatt P, *et al*. 2016. Species- rich and polyploid-poor: insights into the evolutionary role of whole-genome duplication from the Cape flora biodiversity hotspot. *American Journal of Botany* 103:1336–1347

Parisod C, and Broennimann O. 2016. Towards unified hypotheses of the impact of polyploidy on ecological niches. *New Phytologist* 212:540–542

Pécrix Y, Rallo G, Folzer H, *et al*. 2011. Polyploidization mechanisms: temperature environment can induce diploid gamete formation in *Rosa* sp. *Journal of Experimental Botany* 62:3587–3597

Pellicer J, Hidalgo O, Dodsworth S, *et al*. 2018. Genome size diversity and its impact on the evolution of land plants. *Genes* 9:88

Pellicer J, and Leitch IJ. 2020. The Plant DNA C-values database (release 7.1). *The New Phytologist* 226:301–305

Porturas LD, Anneberg TJ, Curé AE, *et al*. 2019. A meta-analysis of whole genome duplication and the effects on flowering traits in plants. *American Journal of Botany* 106:469–476

Ramsey J. 2011. Polyploidy and ecological adaptation in wild yarrow. *Proceedings of the National Academy of Sciences of the United States of America* 108:7096-7101

Ramsey J, Schemske DW. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29:467–501

Ramsey J, Ramsey TS. 2014. Ecological studies of polyploidy in the 100 years following its discovery. *Philosophical Transactions of the Royal Society B* 369:20130352.

Rice A, Šmarda P, Novosolov M, *et al*. 2019. The global biogeography of polyploid plants. *Nature Ecology & Evolution* 3:265–273

Saito K. 1975. Studies on the occurrence of polyploidy and its contributions to the flower plant breeding. XII. On the nature and sterility in the spontaneous triploid cultivars of *Rhodohypoxis baurii* Nel. *Japanese Journal of Plant Breeding* 25:355–362

Schönswetter P, Lachmayer M, Lettner C, *et al*. 2007. Sympatric diploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps are separated along an altitudinal gradient. *Journal of Plant Research* 120:721–725

Sobel JM, Chen GF, Watt LR, *et al*. 2010. The biology of speciation. *Evolution* 64:295–315

Soltis DE, Soltis PS. 1999. Polyploidy: recurrent formation and genome evolution. *Trends in Ecology and Evolution* 14:348–352

Stebbins GL. 1938. Cytological characteristics associated with the different growth habitats in the dicotyledons. *American Journal of Botany* 25:189–198

Stutz HC, and Sanderson SC. 1983. Evolutionary studies of *Atriplex*: chromosome races of *A. confertifolia* (shadscale). *American Journal of Botany* 70:1536–1547

te Beest M, Le Roux JJ, Richardson DM, *et al*. 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* 109:19–45

Thompson KA, Husband BC, Maherali H. 2014. Climatic niche differences between diploid and tetraploid cytotypes of *Chamerion angustifolium* (Onagraceae). *American Journal of Botany* 101:1868 –1875

van Mazijk R, West AG, Verboom GA, *et al*. 2024. Genome size variation in Cape schoenoid sedges (Schoeneae) and its ecophysiological consequences. *American Journal of Botany*, p.e16315

Van de Peer Y, Mizrachi E, Marchal K. 2017. The evolutionary significance of polyploidy. *Nature Reviews Genetics* 18:411–424

Van de Peer Y, Ashman TL, Soltis PS, *et al*. 2021. Polyploidy: an evolutionary and ecological force in stressful times. *The Plant Cell* 33:11–26

Veselý P, Šmarda P, Bureš P, *et al*. 2020. Environmental pressures on stomatal size may drive plant genome size evolution: evidence from a natural experiment with Cape geophytes. *Annals of Botany* 126:323–330

Visger CJ, Germain-Aubrey CC, Patel M, *et al*. 2016. Niche divergence between diploid and autotetraploid *Tolmiea*. *American Journal of Botany* 103:1396–1406

Walczyk AM, Hersch-Green EI. 2019. Impacts of soil nitrogen and phosphorus levels on cytotype performance of the circumboreal herb *Chamerion angustifolium*: implications for polyploid establishment. *American Journal of Botany* 106:906–921

Wei N, Cronn R, Liston A, *et al*. 2019. Functional trait divergence and trait plasticity confer advantage in heterogenous environments. *New Phytologist* 221:2286–2297

Wei N, Du Z, Liston A, *et al*. 2020. Genome duplication effects on functional traits and fitness are genetic context and species dependent: studies of synthetic polyploid *Fragaria*. *American Journal of Botany* 107:262–272

Wu J, Wang M, Zhu Z, *et al*. 2022. Cytogeography of the East Asian Tulips (*Amana*, Liliaceae). *Taxonomy* 2:145–159

Yang C, Zhao L, Zhang H, *et al*. 2014. Evolution of physiological responses to salt stress in hexaploid wheat. *Proceedings of the National Academy of Sciences of the United States of America* 111:11882–11887

Yu F, Kress WJ, Gao J. 2010. Morphology, distribution and chromosome counts of two varieties of *Hedychium villosum* (Zingiberaceae). *Journal of Systematics and Evolution* 48:344–349

Zozomová-Lihová J, Malánová-Krásná I, Vít P, *et al*. 2015. Cytotype distribution patterns, ecological differentiation, and genetic structure in a diploid-tetraploid contact zone of *Cardamine amara*. *American Journal of Botany* 102:1380–1395

Zhang XY, Hu CG, Yao JL. 2010. Tetraploidization of diploid *Dioscorea* results in activation of the antioxidant defense system and increased heat tolerance. *Journal of Plant Physiology* 167:88–94

Table 1.1 Studies where polyploids generally occurred at lower elevations and/or latitudes than their diploid progenitors or where there was no significant relationship between ploidy level distribution and elevation. The information presented in the table (species/genus and locality) was collated from these studies because they showed that polyploids occurred at lower elevations and/or latitudes in plant taxa occurring in different geographic regions across the globe.

Species/genus	Locality	Reference
Dicerothamnus rhinocerotis	South Africa	Chumová *et al*. (2023)
Festuca amethystina	Central and South-Eastern Europe	Kiedrzyński *et al*. (2021)
Amana	China, Japan and Korean Peninsula	Wu *et al*. (2022)
Senecio carniolicus	Eastern Alps	Schönswetter *et al*. (2007)
Centaurea jacea complex	Belgian Ardennes	Hardy *et al*. (2000)
Linum suffruticosum	South-western Europe and Morocco	Afonso *et al*. (2021)
Isoetes	East Asia	Liu *et al*. (2004)
Turnea sidoides subsp. *pinnatifida*	Central Argentina	Elías *et al*. (2011)
Chamerion angustifolium	North America, Greenland, and Iceland	Thompson *et al*. (2014)
Atriplex confertifolia	The Great Basin	Stutz and Sanderson (1983)
Polylepis australis	Argentina	Kessler *et al*. (2014)
Seseli libanotis	Central Europe and adjacent areas	Němečková *et al*. (2018)
Artemisia sieberi	Iran	Jalili *et al*. (2013)
Thymus section *Mastichina*	Iberian Peninsula	Girón *et al*. (2012)
Brachypodium distachyon	Iberian Peninsula	Manzaneda *et al*. (2012)
Erysimum mediohispanicum	Iberian Peninsula	Muñoz-Pajares *et al.* (2017)

Figure Legend

Fig. 1.1 A flowering *Rhodohypoxis baurii* var. *platypetala* plant with a white flower that is pale pink in the outside, in the KwaZulu-Natal province, South Africa.

Fig. 1.2 Flowering *Rhodohypoxis baurii* var. *platypetala* plants in a wild population in the KwaZulu-Natal province, South Africa. The plants were found growing in between rocks.





CHAPTER 2

ELEVATION AND ASSOCIATED ENVIRONMENTAL VARIABLES ARE WEAK PREDICTORS OF BROAD-SCALE POLYPLOID DISTRIBUTION

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Prepared for submission to *New Phytologist*

Abstract

Polyploidy is a prominent evolutionary force with a demonstrated potential to confer an advantage for plants under different environmental conditions. Polyploid outcomes in plants may result in ecological divergence between polyploids and their progenitors, although collective findings from studies have been inconsistent in their association of environmental variables with polyploid distribution. In previous work, climate, specifically temperature, appeared to strongly drive global polyploid distribution, yet its effect was indirect and mediated by increased polyploidy in perennial herbs. Moreover, a long-standing hypothesis that polyploids generally occupy higher elevations than diploids has been inconsistent at local scales and negligible at a global scale. The effect of elevation on polyploid distribution is likely scale-dependent and potentially influenced by mountain ranges across geographic regions. To ascertain if consistent elevational segregation exists between diploid and polyploid plants at a broad spatial scale (within geographic regions), we quantified within-study effect sizes of elevation on the distribution of diploid and polyploid populations from a dataset of 28 studies consisting of > 2000 populations. We found negative to small effect sizes in 20 studies and only five studies showing larger effect sizes, indicating that polyploid populations were mostly found at lower elevations than diploids. Second, we compared daily data of climatic and soil variables between the highest diploid and highest polyploid elevations across the studies and found that in general, diploids occurred at higher elevations than polyploids whereas polyploids experienced a higher temperature range and more rainfall. We then used a regression model to examine whether the climatic and soil variables predicted the presence of polyploids at the highest elevations across these studies. Geographic region and decreasing soil moisture were the most important predictors of polyploid presence at high elevations. However, the total variance explained by the overall model was very low for all variables (0.3%). We conclude that evidence for elevation

segregation between diploid and polyploid plants, when present, is likely confounded by factors such as geographic region and historical contingency, with a possibility that potential differences in environmental variables between diploid and polyploid populations are likely describing the sampled populations and not necessarily predicting polyploid distribution. It is also more useful to compare environmental variables between diploid and polyploid populations from the same species than only looking at polyploid species across the globe.

Key words: Elevation, effect size, meta-analysis, polyploidy, soil moisture

INTRODUCTION

Polyploidy is generally considered an important mode of angiosperm ecology and evolution (for review, see Husband *et al*., 2013; Ramsey and Ramsey, 2014; Van de Peer *et al*., 2017). Recently, polyploids were found to occur at higher latitudes, with polyploid distribution increasing towards the Northern Hemisphere (Ehrendorfer, 1980; Brochmann *et al*., 2004; Rice *et al*., 2019; Bureš *et al*., 2024). Moreover, polyploids are associated with changing or stressful environments owing to, for instance, increased genetic variation, novel traits, changes in gene expression, enhanced interaction with mutualists, and increased adaptive potential (Ramsey and Ramsey, 1998; Husband *et al*., 2013; Lohaus and Van de Peer, 2016; Van de Peer *et al*., 2017, 2021; Bomblies, 2020). Environmental factors such as cold temperatures are also associated with the formation of unreduced gametes (Ramsey and Ramsey, 1998; Van de Peer *et al*., 2017). Such trends in the global distribution of polyploids or general association of polyploids with environmental change or stress suggest that polyploid distribution is potentially driven by environmental variables.

Factors such as life-history traits (e.g., increased instances of polyploidy in perennial herbs) were found to have a direct effect on polyploid distribution while climate, specifically temperature, had an indirect relationship with global polyploid distribution (Rice *et al*., 2019). Consequently, whether polyploidy drives general ecological divergence between diploid and polyploid plants remains unresolved, especially because broad climatic niches have been found to be shared between diploid and polyploid plants (Glennon *et al*., 2014). Polyploid distribution is also expected to be limited in nutrient-poor environments due to the higher demands for nitrogen and phosphorus in polyploids after whole genome duplication (Leitch and Leitch, 2008; Šmarda *et al*., 2013; Guignard *et al*., 2016; Faizullah *et al*., 2021), yet the effect of phosphorus soil availability on global polyploid distribution was found to be negligible (Rice *et al*., 2019). At local scales, increased nitrogen and phosphorus do not

always correspond to higher performance by polyploid plants in different species potentially due to smaller genomes that do not have high nutrient requirements (e.g., Harms *et al*., 2021; but see Guignard *et al*., 2016; Walczyk and Hersch-Green, 2019; Annerberg and Segraves, 2020). It is apparent that the relationship between environmental variables and polyploidy varies for plant systems and at different spatial scales.

Mountain climates often present considerable climate and soil heterogeneity (Grab, 2013; Carbutt and Edwards, 2003, 2006, 2015) where diploid and polyploid plants may shift their distribution to segregate into different elevations (Husband *et al*., 2013). Recently, a relatively high frequency (39%) of polyploids was found in montane grasslands (Rice *et al*., 2019) which are characterised by high elevations (World Wildlife Fund), suggesting that polyploid plants tend to generally populate higher elevations than diploids (Husband *et al*., 2013). We might think of elevation as a composite variable that is highly correlated with changes in environmental variables such as temperature and precipitation. As a result, elevation can be used as a primary variable as it represents other variables. For example, elevation differences were associated with lapse rates in hourly temperature data where temperature decreased with increasing elevation (e.g., Grab, 2013). Collective results from studies have shown an inconsistent trend in the relationship between polyploid distribution and vertical distance, with polyploids often occurring at higher elevations than diploids, but in some instances, at lower elevations than diploids (Elías *et al*., 2011; Zozomová-Lihová *et al*., 2015; Kudo and Hirao, 2020; Kiedrzyński *et al*., 2021), and at a global scale, elevation had a weak relationship with polyploid distribution (Rice *et al*., 2019). Possible explanations for this inconsistent or lack of association with elevation are that polyploid distribution may be related to other variables such as perenniality and species richness (Rice *et al*., 2019) and environmental stressors and the subsequent formation of unreduced gametes (Van de Peer *et al*., 2017, 2021), rather than a trend towards high or low elevations. Moreover, the general

trend of polyploids occurring at high elevations may also be influenced by factors such as limited sampling of diploid populations compared to polyploid populations, and different elevation gradients in different geographic regions where different ploidies occur.

Comparisons of elevation between diploid and polyploid populations in different studies provide a useful opportunity to test if polyploids are consistently found at higher elevations than diploids, and an opportunity to refine general comparisons of polyploid distribution based on elevation and associated environmental variables which may provide trends that are unique for different plant systems. Here, we used a meta-analysis approach to assess if within-study effect size direction and magnitude of the difference in elevation between polyploid and related diploid populations were consistent for different studies – i.e., are polyploids consistently found in higher elevations than diploids across studies? We hypothesized that climate (e.g., temperature) and soil variables likely facilitate the distribution of ploidy levels, rather than elevational differences themselves. We then examined whether elevation and associated environmental variables (climate and soil) were strong predictors of broad-scale polyploid distribution. We also explored whether environmental variables differ at the highest elevations where diploids and polyploids of the same species occur.

METHODS

Literature search approach

We performed searches in the Web of Science (Science Citation Index Expanded, 2012–2023) and Google Scholar databases for literature that would address our questions using the following key words (search conducted in November 2023): ‘polyploid distribution’, ‘cytogeography’, and ‘cytotype distribution’. To broaden our sample, we did not filter our searches by geographic region. We assessed 85 candidate studies from Google

Scholar and 133 studies from Web of Science, and we included studies that were included in the broad-scale analysis of polyploid distribution by Glennon *et al*. (2014). The following criteria were used to assess studies that were relevant to answer our questions: 1) did the study focus on plants, 2) contained ploidy level estimates from flow cytometry and/or chromosome count data, 3) presence of both diploid and polyploid populations regardless of the type of polyploidy (auto-/allopolyploidy) and not only mixed-ploidy populations, 4) exclusively wild populations, 5) accessibility of elevation data, and 6) availability of precise population location information.

Our calculation of effect sizes of polyploid distribution by elevation (see details below) required both diploid and polyploid populations to be present in a study. We excluded studies that contained higher ploidy populations and no diploids (e.g., Duchoslav *et al*., 2013; Wefferling *et al*., 2017; Castro *et al*., 2018; Decanter *et al*., 2020). Some studies focused on fewer diploid and polyploid populations compared to the other studies (e.g., Baack and Stanton 2005; Raabová *et al*., 2008) but they were considered if they met all the other requirements above. Studies focusing on animals (e.g., Zhong *et al*., 2019; Shields and Procunier, 2019) were excluded. Review papers were also excluded as they did not contain data but summarised key findings and trends from previous work (e.g., Weiss-Schneeweiss *et al*., 2013). After the removal of duplicates, review papers, and studies that did not meet criteria, 28 studies consisting of 2659 populations were retained for subsequent analyses. These studies focused on different plant systems and contained enough data to answer our questions regarding ploidy level distribution based on environmental variables. Our sampled studies span different regions such as the Greater Cape Floristic Region (Krejčíková *et al*., 2013), Sino-Japanese Floristic Subregion (Wu *et al*., 2022), the Western Carpathians (Wos *et al*., 2019) and Belgian Ardennes (Hardy *et al*., 2000) as well as different biomes such as the

Mediterranaean forests, woodlands and scrub (Fig. 2.1; for categories of biomes, see Olson *et al*., 2001).

Qualitative assessment of ploidy level distribution

We examined whether the reporting of the environmental distribution of diploids and polyploids was consistent across the sampled studies. From each study, we reported a summary of key findings regarding ploidy level distribution based on environmental variables (Table 2.1).

Effect sizes of elevation based on means

To assess the consistency of ploidy level distribution based on elevation across studies, we calculated an appropriate effect size (Hedge's *g*, standardized mean difference; Borrenstein *et al*., 2011) from the extracted elevation data per study where the standardised mean difference was calculated as [(mean1 – mean2)/overall standard deviation)]. We used a single measure of effect size for comparability of results across studies. Our effect size direction per study considered two groups as the experimental treatments (polyploid populations including all ploidy levels higher than diploids, mean1) minus the control treatment (diploid populations, mean2) where a negative effect size would indicate that polyploids generally occurred at lower elevations than diploids. For mixed-ploidy populations containing diploid and polyploid individuals, we included these populations both in the diploid and polyploid experimental treatments. To show the potential dispersion in the effect sizes, we did not calculate a summary effect size (i.e., weighted mean of the individual effects) because the studies focused on different plant systems and represented different geographic regions. The number of diploid and polyploid populations also differed across the studies, so a summary effect size would be biased in that studies with larger sample sizes would produce an effect size that is close to the summary effect size due to lower standard

error, presenting misleading interpretations. We assessed the effect sizes per study based on the benchmark values to represent small (0.2), medium (0.5), and large (0.8) effect sizes (Cohen, 1988). We found that some studies referred to vertical distance as elevation while others used altitude, so we refer to vertical distance as 'elevation' for consistency (McVicar and Körner, 2013). Note that studies focusing on mountain ranges such as the present study should refer to elevation, not altitude, where the reference point is usually mean sea level (McVicar and Körner, 2013).

Polyploid distribution and environmental correlates

To further assess the potential segregation between diploids and polyploids at different elevations across studies, we extracted environmental data (see specific details below) for the highest diploid and highest polyploid elevations per study (Fig. 2.1). Our use of diploid and polyploid populations at the highest elevations per study enabled us to also potentially counteract spatial autocorrelation and account for the observed differences in the number of populations sampled across studies, but we acknowledge that some regions (e.g., Africa) were underrepresented in our meta-analysis possibly due to limited polyploid taxa or low sampling efforts (Fig. 2.1). Further, using the highest elevations enabled us to test whether our focal variable (elevation) and associated environmental variables predicted polyploid distribution, without overall influence from localities where diploids and polyploids potentially occupy similar elevations. Daily data for 13 parameters ('environmental variables' hereafter; Fig. S1) were downloaded in November 2023 in POWER Project's Hourly 2.0.0 version 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 10 years (2012–2022).

To reduce the number of correlated environmental variables, we used the R package *corrplot* (version 0.92; Wei and Simko, 2017) to construct a correlation matrix where we excluded variables using a coefficient threshold not exceeding 0.70 (Fig. S1). Finally, six environmental variables were assessed for possible relationships with the presence of polyploids at high elevations across studies: 1) profile soil moisture, where 0 and 1 indicate water-free soil and a completely saturated soil, respectively, 2) temperature range at 2 m above Earth surface (ºC), 3) relative humidity (%), 4) downward thermal infrared irradiance (horizontal infrared radiation intensity; W m^{-2}), 5) ultraviolet (UV) radiation exposure index (dimensionless), and 6) total precipitation (mm per day). We also tested if latitude and elevation at the highest diploid and highest polyploid elevations significantly differed across sampled studies. To counteract the differences in the reported elevation data across studies, where the data were either reported in meters or meters above sea level and likely recorded using different data instruments or extracted from different data products, we elected to use the elevation data extracted from NASA's POWER project (https://power.larc.nasa.gov/data-access-viewer/) for comparability and consistency as these data could be extracted from the localities for each population using GPS coordinates, independent of the reported data per study. Kruskal-Wallis tests were used to compare each variable between diploids and polyploids across studies and among geographic regions. We included the variables into a Principal Component Analysis (PCA) to explore their association with the lowest and highest elevations across studies. We used the Spearman rank-correlation coefficient to test the correlation between the variables and the highest diploid and highest polyploid elevations as well as lowest diploid and lowest polyploid elevations across studies. We acknowledge that it is more useful to compare diploid and polyploid populations from the same species. Consequently, we selected a study (*Cardamine amara*; Zozomová-Lihová *et al*., 2015) that produced a large effect size with high precision (i.e., narrow confidence interval; see effect

sizes in the results section) to include in further analyses where we explored the association of the extracted environmental variables with diploids and polyploids of the same species at the highest elevations.

Last, we used a binomial generalized linear model (GLM) using a logit link function to assess the effect of the variables on ploidy level distribution at high elevations across studies where diploids were considered as 'absence of polyploids' (0) and polyploids as 'present' (1). Geographic region was included in the model as a covariate. We used the packages *pscl* (version 1.5.5.1; Jackman, 2020) and *domir* (version 1.1.1; Luchman, 2023) to compute the overall fit of the GLM as well as the relative importance of each variable. All statistical analyses were conducted in R (version 4.2.1; R Core Team, 2022).

RESULTS

Qualitative assessment of ploidy level distribution reported in previous work

In our assessment, we found that previous studies considered different environmental variables such as temperature, precipitation parameters, vegetation type, and soil aridity as well as soil moisture and their possible relationships with polyploid distribution (Table 2.1). Niche overlap, where environmental variables and distribution did not differ between diploids and polyploids, was found in 12 studies, and the segregation between diploids and polyploids based on environmental variables was supported by 12 studies (Table 2.1). There was no consistent pattern on the effect of environmental variables on polyploid distribution, and the segregation between diploids and polyploids based on environmental variables found in 12 studies was not explained by the same variables. Temperature was the most influential predictor of polyploid distribution for five studies, whereas soil moisture, vegetation type, and precipitation parameters explained polyploid distribution for the rest of the studies (Table 2.1). In our assessment, niche overlap between diploids and polyploids was found for

different plant systems and regions including *Arabidopsis arenosa* in the Western Carpathians, *Thymus* section *Mastichina* in the Iberian Peninsula, and *Centaurea jacea* in the Belgian Ardennes (Table 2.1). There was also no apparent pattern of niche overlap or niche differentiation by biomes. For instance, two different studies were conducted in the temperate coniferous forests of the Pacific Northwest of North America, but niche overlap for diploids and polyploids was found in *Heuchera cylindrica* (Godsoe *et al*., 2013) and niche differentiation between ploidies was found in *Tolmiea* (Visger *et al*., 2016; Table 2.1).

The range of ploidies differed across studies, with 18 studies focusing on diploids and tetraploids whereas octoploids represented the highest ploidy level recorded in four studies (Table 2.1). Closely related taxa are not over represented in the analyses relative to unrelated groups (Table 2.1). Consequently, there did not appear to be a phylogenetic effect on the results. For example, in the family Saxifragaceae, work on *Heuchera* found niche overlap between ploidy levels whereas niche differentiation was found in *Tolmiea* (Table 2.1).

Effect sizes of elevation based on means

We found differences in effect size direction of elevation. Twelve of the total 28 studies had negative effect sizes (Fig. 2.2). Only five studies had large effect sizes [largest effect size (ES) = 2.70; 95% CI = 1.48 to 3.92], three studies had moderate effect sizes (highest moderate ES = 0.78; 95% CI = 0.41 to 1.15), and 20 studies showed negative to low effect sizes (ES < 0.5; Fig. 2.2). When considering the upper bound 95% conference intervals, 13 studies had negative to small effect sizes (Fig. 2.2). There was no clear clustering of effect sizes by geographic regions (Fig. 2.2).

Environmental correlates associated with elevation differences

A summary of the variance in the environmental variables across studies showed that diploids tended to live in a broader soil moisture range at higher elevations while polyploids

tended to experience a higher temperature range and higher precipitation conditions (Fig. 2.3D, F, H). General comparisons of variables across studies did not yield representative results for all regions. In the North American studies, elevation did not significantly differ between diploids and polyploids; in European studies, diploids reached higher elevations than polyploids potentially due to refugia during the ice age; in Asian regions, polyploids reached higher elevations than diploids (Fig. 2.4B). In Asian regions, precipitation did not differ between diploids and polyploids (Fig. 2.4E). Polyploids in Asian regions reached the lowest and highest elevations recorded for this study (Fig. 2.4B).

The first two principal components (PC1 and PC2) cumulatively explained 68.19% of the variance in the six environmental variables at the highest and lowest elevations where diploids and polyploids occurred across studies (Fig. 2.5; Table S1). However, there was no clear segregation in the environmental variables by high and low elevations (Fig. 2.5) and the variables were significantly correlated with both the highest and lowest elevations across studies where diploids and polyploids occurred (Table 2.2). These findings might be potentially explained by the discordance in the elevation ranges across studies where a 'high' elevation in a study could potentially represent a 'low' elevation in another study (Fig. 2.4B). The PCA at a study level also showed that there was overlap in environmental variables at the highest elevations where diploids and polyploids of *Cardamine amara* were found (Fig. 2.6).

Polyploid distribution and environmental correlates

Our GLM findings suggest that polyploid presence was more likely at high elevations across studies with the increase in temperature range, humidity, and horizontal infrared radiation intensity (Table 2.3). Geographic region was the most important predictor for polyploid distribution (R^2_{MF} = 46%; $P < 0.0001$), likely due to the overrepresentation of some

regions in the data compared to other regions. Low soil moisture was the second important predictor of where polyploid presence was more likely (R^2_{MF} = 28%; $P < 0.0001$). Relative humidity (R^2_{MF} = 12%, $P < 0.0001$) and temperature (R^2_{MF} = 7%, $P < 0.0001$) were the third and fourth contributing variables, respectively, with precipitation being the least contributing variable (R^2_{MF} = 1%; $P = 0.001$; Table 2.3). The correlation coefficients between relative humidity, temperature, and precipitation, and the most influential environmental variable, soil moisture, were 0.58, -0.50, and 0.19, respectively, and they were all less than the coefficient threshold of 0.70 (see Fig. S1). The total variance explained by the overall model was 0.3% (Table 2.3).

DISCUSSION

A broad association between polyploidy and environmental variables presents myriad challenges for outlining a unifying hypothesis for polyploid biogeography. For instance, previous work did not find general ecological divergence between diploids and polyploids (Glennon *et al*., 2014). Climate-mediated drivers of polyploid distribution were also not found for global polyploid distribution (Rice *et al*., 2019). One challenge in polyploid biogeography is that many polyploids are associated with mountain systems. Mountain climates provide considerable environmental heterogeneity (Carbutt and Edwards, 2003, 2006, 2015; Grab, 2013) where there may be potential ploidy level segregation by elevation and associated environmental variables (Husband *et al*., 2013). Rice *et al*. (2019) recently showed a latitudinal trend where global polyploid frequency increased away from the equator, but elevation was found to have a weak relationship with polyploid distribution, likely due to the coarse scale of the study. Here, we used a meta-analysis approach to examine if populations of different ploidy levels consistently differed in elevation and environmental habitats within and across 28 studies representing different plant systems and

geographic regions. Our qualitative assessment showed that both niche differentiation (e.g., Visger *et al.,* 2016; Rojas-Andrés *et al*., 2020) and niche overlap (e.g., Godsoe *et al*., 2013; Kobrlová *et al*., 2022) were reported for diploids and polyploids in different studies. For instance, Manzaneda *et al*. (2012) found that polyploid distribution was associated with aridity (diploids in less arid vs polyploids in more arid biomes), Zozomová-Lihová *et al*. (2015) found that the likelihood of polyploid distribution decreased with increasing temperature, Kiedrzyński *et al*. (2021) found that the most influential predictor for polyploid distribution was mean temperature of the driest quarter (e.g., more polyploids if dry habitats), whereas in other studies, no evidence was found for niche divergence between diploids and polyploids or the association between polyploid distribution and environmental variables (e.g., Girón *et al*., 2012; Kessler *et al*., 2014; Kolář *et al*., 2016; Wos *et al*., 2019). These studies generally support that polyploid distribution is not always associated with key environmental variables, like temperature or precipitation, and that general ecological divergence does not always exist between polyploids and their diploid progenitors (e.g., Glennon *et al*., 2014; Castro *et al*., 2019).

It appears that the association of environmental variables with polyploid distribution is scale-dependent and likely species-specific and population-specific. For instance, polyploids are hypothesized to occur at high elevations and latitudes as well as certain biomes, potentially due to mechanisms such as plasticity and increased heterozygosity (Leitch and Leitch, 2008; Husband *et al*., 2013) and the formation of unreduced gametes in extreme cold temperatures (Van de Peer *et al*., 2017), yet the general trend of polyploids occurring at higher elevations than diploids is inconsistent at local scales and is negligible at a global scale. Here, we found that the effect size of elevation on polyploid distribution was not consistently large or positive across 28 studies, indicating that polyploid populations generally occurred at lower or similar elevations to their progenitors. The effect sizes did not

show a clear pattern of clustering by geographic regions. These results are supported by collective findings from studies where the association of elevation with polyploid distribution was inconsistent. For instance, polyploids have been found to occur at higher elevations than diploids (e.g., Krejčíková *et al*., 2013) as well as at lower elevations than diploids in different plant systems (e.g., Kiedrzyński *et al*., 2021; Chumová *et al*., 2023).

We acknowledge that environmental correlates associated with elevation differences may influence polyploid distribution, rather than a ploidy level trend towards high or low elevations. Latitude and elevation may be considered 'composite' variables as these two variables are often strongly correlated with changes in other environmental variables like temperature, precipitation, and soil moisture (e.g., Bureš *et al*., 2024). The populations evaluated in our study, especially the European studies, appear to have reached high latitudes (~50N) and associated lowest temperatures in the coldest quarter where plants with higher ploidy levels would have responded to corresponding changes in the environment, such as freezing temperatures (Bureš *et al*., 2024). To test this possibility, we assessed if climatic and soil variables differed at the highest diploid and highest polyploid elevations across studies. A clear trend in our findings was that at the recorded high elevations across the sampled studies, diploids tended to live in a broader range of soil moisture measures whereas polyploids appeared to occur in sites with larger ranges in temperature and higher amounts of rainfall.

General comparisons of environmental variables between diploid and polyploid populations such as our findings above are likely describing population differences in environmental variables, rather than predictors or drivers of polyploid distribution per se. We then used a regression model to test for relationships between environmental variables and polyploid presence at the highest elevations across studies to account for the potentially population-specific findings. We found that all variables considered in this study were

significantly correlated with high elevations across studies where diploids and polyploids occurred, and that geographic region significantly influenced the findings. The total variance explained by the overall model in the present study was negligible (0.3%), suggesting that while all variables were significantly correlated with high elevations across studies, the variables were not strong predictors of polyploid distribution at higher elevations than diploids. We also found that the variables were significantly correlated with both the highest and lowest elevations across studies. However, of the environmental variables examined, decreasing soil moisture was the most important predictor for the presence of polyploids at high elevations (R^2_{MF} = 28%; $P < 0.0001$), which coincides with studies where polyploid distribution was associated with dry habitats (e.g., Elías *et al*., 2011; Manzaneda *et al*., 2012). This general association of low soil moisture and polyploid distribution is not representative of all plant systems used in our study. For instance, in *Veronica* subsection *Pentasepalae* (Rojas-Andrés *et al*., 2020), polyploids generally occurred in wetter habitats than diploids.

Studies focusing on mountain environments can benefit from small prediction errors by working at small scales (e.g., within slopes) to capture the complex association of elevation with corresponding environmental variables (e.g., Grab, 2013). Work on different mountain ranges generally overlook the complexity and variability of the association between polyploid distribution and environmental variables, resulting in incomparable findings to local-scale studies and putative confounding factors such as different slopes within geographic ranges which are not accounted for. The findings presented in this study indicate that climatic and soil variables are weak predictors for general ploidy level distribution patterns. In previous work, polyploid distribution was directly explained by the presence of perennials, rather than a climate-mediated polyploid distribution (Rice *et al*., 2019). In our study, all but six taxa from the sampled studies are perennial (annual = 1, biennial–perennial = 4, annual–perennial = 1), so our results are unlikely due to the effect of life history traits.

Habitat availability and subsequent low species competition can also facilitate ploidy level distribution (e.g., Kiedrzyński *et al*., 2021). We also acknowledge different modes of polyploidy (auto/allopolyploidy) or the lack of historical component which prevents us from inferring underlying causes of ploidy level distribution (e.g., Pleistocene refugia).

Our results indicate that comparing diploid and polyploid populations from the same species is more useful than looking at polyploid species across the globe. Indeed, we found that geographic region significantly influenced our findings, although we acknowledge that some regions (e.g., Europe) were overrepresented in the data compared to other regions. At the taxon level, we found that there was overlap in environmental variables at the highest elevations where diploids and polyploids of *Cardamine amara* occurred, indicating that ploidy levels likely experience similar environmental conditions at high elevations.

Together, our present findings did not support that the environmental correlates associated with elevation coincide with broad-scale polyploid distribution, or that elevation and associated environmental variables have a consistent effect on ploidy level distribution. Our qualitative assessment of previous work for ploidy level distribution based on environmental variables demonstrated discordance across studies. We conclude that although necessary for identifying emerging and insightful trends in polyploid biology, work at large scales such as the one presented here (e.g., across biomes and geographic regions, or at a global level) do not necessarily explain or predict unique polyploid outcomes for different plant systems and populations. For instance, due to higher demands for nitrogen and phosphorus after whole genome duplication, polyploids may potentially perform better under nutrient enrichment than diploids (Guignard *et al*., 2016; Walczyk and Hersch-Green, 2019; Annerberg and Segraves, 2020), yet previous work found no strong association between global polyploid distribution and soil phosphorus availability likely due to the coarse scale of the soil phosphorus data (Rice *et al*., 2019). Also, shared niches were found for diploid and

polyploid plants at broad scales (Glennon *et al*., 2014), yet niche differentiation exists in different plant systems at local scales (e.g., Visger *et al.,* 2016; Rojas-Andrés *et al*., 2020). In conclusion, polyploid outcomes are unlikely to be uniform across different plant systems. Our findings showed that cytogeographic studies of many mixed-ploidy species showed niche overlap between diploids and polyploids. Experimental work (e.g., growth chamber and reciprocal transplant experiments) is likely more useful to complement cytogeographic studies and to establish if an association between ploidy level distribution and environmental conditions exists.

DATA AVAILABILITY

Data available at OSF link

Literature Cited

Anneberg TJ, Segraves KA. 2020. Nutrient enrichment and neopolyploidy interact to increase lifetime fitness of *Arabidopsis thaliana*. *Plant and Soil* 456:439–453

Baack EJ, and Stanton ML. 2005. Ecological factors influencing tetraploid speciation in snow buttercups (*Ranunculus adoneus*): niche differentiation and tetraploid establishment. *Evolution* 59:1936–1944

Bomblies K. 2020. When everything changes at once: finding a new normal after genome duplication. *Proceedings of the Royal Society B* 287:20202154

Borenstein M, Hedges LV, Higgins JPT, *et al*. 2011. Introduction to meta-analysis. Chichester, West Sussex, United Kingdom: John Wiley & Sons

Brochmann C, Brysting AK, Alsos IG, *et al*. 2004. Polyploidy in arctic plants. *Biological Journal of the Linnean Society* 82:521–536

Bureš P, Elliott TL, Veselý P, *et al*. 2024. The global biogeography of angiosperm genome size is shaped by climate and range size. *New Phytologist* 242:744–759

Castro M, Castro S., Figueiredo A, *et al*. 2018. Complex cytogeographical patterns reveal a dynamic tetraploid–octoploid contact zone. *AoB Plants* 10:ply012

Castro M, Loureiro J, Serrano M, *et al*. 2019. Mosaic distribution of cytotypes in a mixed-ploidy plant species, *Jasione montana:* nested environmental niches but low geographic overlap. *Botanical Journal of the Linnean Society* 190:51–66

Cohen J. 1988. Statistical power analysis for the behavioral sciences. Lawrence Erlbaum Associates, New York

Carbutt C, Edwards TJ. 2003. The flora of the Drakensberg Alpine Centre. *Edinburgh Journal of Botany* 60:581–607

Carbutt C, Edwards TJ. 2006. The endemic and near-endemic angiosperms of the Drakensberg Alpine Centre. *South African Journal of Botany* 72:105–132

Carbutt C, Edwards TJ. 2015. Plant-soil interactions in lower-upper montane systems and their implications in a warming world: a case study from the Maloti-Drakensberg Park, southern Africa. *Biodiversity* 16:262–277

Chumová Z, Monier Z, Šemberová K, *et al*. 2023. Diploid and tetraploid cytotypes of the flagship Cape species *Dicerothamnus rhinocerotis* (Asteraceae): variation in distribution, ecological niche, morphology and genetics. *Annals of Botany* 133:851–870

Decanter L, Colling G, Elvinger N, *et al*. 2020. Ecological niche differences between two polyploid cytotypes of *Saxifraga rosacea*. *American Journal of botany* 107:423–435

Dobeš C, Rossa J, Paule J, *et al*. 2013. Morphology, DNA-molecular variation, karyology, ecogeography, and phytosociology suggest allopatric differentiation and species rank for *Potentilla rigoana* (Rosaceae). *Taxon* 62:733–745

Duchoslav M, Šafářová L, and Jandová M. 2013. Role of adaptive and non-adaptive mechanisms forming complex patterns of genome size variation in six cytotypes of polyploid *Allium oleraceum* (Amaryllidaceae) on a continental scale. *Annals of Botany* 111:419–431

Ehrendorfer F. 1980. Polyploidy and distribution. In *Polyploidy: Biological Relevance* 45–60. Springer Boston

Elías G, Sartor M, Solís Neffa VG. 2011. Patterns of cytotype variation of *Turnera sidoides* subsp. *pinnatifida* (Turneraceae) in mountain ranges of central Argentina. *Journal of Plant Research* 124:25–34

Faizullah L, Morton JA, Hersch-Green EI, *et al*. 2021. Exploring environmental selection on genome size in angiosperms. *Trends in Plant Science* 26:1039–1049

Girón V, Garnatje T, Vallès J, *et al.* 2012. Geographical distribution of diploid and tetraploid cytotypes of *Thymus* sect. *Mastichina* (Lamiaceae) in the Iberian Peninsula, genome size and evolutionary implications. *Folia Geobotanica* 47:441–460

Glennon KL, Ritchie ME, Segraves KA. 2014. Evidence for shared broad-scale climatic niches of diploid and polyploid plants. *Ecology Letters* 17:574–582

Godsoe W, Larson MA, Glennon KL, *et al*. 2013. Polyploidization in *Heuchera cylindrica* (Saxifragaceae) did not result in a shift in climatic requirements. *American Journal of Botany* 100:496–508

Guignard MS, Nichols RA, Knell RJ, *et al*. 2016. Genome size and ploidy influence angiosperm species' biomass under nitrogen and phosphorus limitation. *New Phytologist* 210:1195–1206

Grab SW. 2013. Fine-Scale variations of near-surface-temperature lapse rates in the high Drakensberg Escarpment, South Africa: Environmental Implications. *Arctic, Antarctic, and Alpine Research* 45:500–514

Hardy OJ, Vanderhoeven S, De Loose M, *et al*. 2000. Ecological, morphological, and allozymic differentiation between diploid and tetraploid knapweeds (*Centaurea jacea*) from a contact zone in the Belgian Ardennes. *New Phytologist* 146:281–290

Harms NE, Cronin JT, Gaskin JF. 2021. Increased ploidy of *Butomus umbellatus* in introduced populations is not associated with higher phenotypic plasticity to N and P. *AoB PLANTS* 13:plab045

Husband BC, Baldwin SJ, Suda J. 2013. The incidence of polyploidy in natural plant populations: major patterns and evolutionary processes. *Plant Genome Diversity Volume 2*, I.J. Leitch et al. (eds.), 255–276. Springer Vienna

Jackman S. 2020. pscl: classes and methods for R developed in the Political Science Computational Laboratory. United States Studies Centre, University of Sydney.

Sydney, New South Wales, Australia. R package version 1.5.5.1. https://github.com/atahk/pscl/

Jalili A, Rabie M, Azarnivand H, *et al*. 2013: Distribution and ecological consequences of ploidy variation in *Artemisia sieberi* in Iran. *Acta Oecologica* 53:95–101

Kessler M, Kühn A, Solís Neffa VG, *et al*. 2014. Complex geographical distribution of ploidy levels in *Polylepis australis* (Rosaceae), an endemic tree line species in Argentina. *International Journal of Plant Sciences* 175:955–961

Krejčíková J, Sudová R, Lučanová M, *et al*. 2013. High ploidy diversity and distinct patterns of cytotype distribution in a widespread species of *Oxalis* in the Greater Cape Floristic Region. *Annals of Botany* 111:641–649

Krejčíková J, Sudová R, Oberlander K, *et al*. 2013. The spatio-ecological segregation of different cytotypes of *Oxalis obtusa* (Oxalidaceae) in contact zones. *South African Journal of Botany* 88:62–68

Kiedrzyński M. Zielińska KM, Jedrzejczyk I, *et al*. 2021. Tetraploids expanded beyond the mountain niche of their diploid ancestors in the mixed-ploidy grass *Festuca amethystina* L. *Scientific Reports* 11:18735

Kobrlová L, Duchoslav M, Hroneš M. 2022. Morphological, ecological and geographic differences between diploids and tetraploids of *Symphytum officinale* (Boraginaceae) justify both cytotypes as separate species. *AoB PLANTS* 14:plac028

Kolář F, Lučanová M, Záveská E, *et al*. 2016. Ecological segregation does not drive the intricate parapatric distribution of diploid and tetraploid cytotypes of the *Arabidopsis arenosa* group (Brassicaceae). *Biological Journal of the Linnean Society* 119:673–688

Kudo G, Hirao AS. 2020. Geographical distribution, genetic diversity, and reproductive traits of mixed polyploid populations in *Parasenecio kamtschaticus* (Senecioneae; Asteraceae). *Plant Systematics and Evolution* 306:86

Leitch AR, Leitch IJ. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* 320:481–483

Li J, Wan Q, Guo YP, *et al*. 2014. Should I stay or should I go: Biogeographic and evolutionary history of a polyploid complex (*Chrysanthemum indicum* complex) in response to Pleistocene climate change in China. *New Phytologist* 201:1031–1044

Liu K, Meng W, Zheng L, *et al*. 2019. Cytogeography and chromosomal variation of the endemic East Asian herb *Lycoris radiata*. *Ecology and Evolution* 9:6849–6859

Lohaus R, Van de Peer Y. 2016. Of dups and dinos: evolution at the K/Pg boundary. *Current Opinion in Plant Biology* 30:62–69

Luchman J. 2023. domir: tools to support relative importance analysis. R package version 1.1.1, https://CRAN.R-project.org/package=domir

Manzaneda AJ, Rey PJ, Bastida JM, *et al*. 2012. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *New Phytologist* 193:797–805

Melichárková A, Španiel S, Marhold K, *et al*. 2019. Diversification and independent polyploid origins in the disjunct species *Alyssum repens* from the Southeastern Alps and the Carpathians. *American Journal of Botany* 106:1499–1518

McVicar TR, Körner C. 2013. On the use of elevation, altitude, and height in the ecological and climatological literature. *Oecologia* 171:335–337

Němečková H, Krak K, Chrtek J. 2018. Complex pattern of ploidal and genetic variation in *Seseli libanotis* (Apiaceae). *Annales Botanici Fennici* 56:57–77

Olson DM, Dinerstein E, Wikramanayake ED, *et al*. 2001. Terrestrial ecoregions of the world: A new map of life on Earth: A new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *BioScience* 51:933–938

Raabová J, Fischer M, and Münzbergová Z. 2008. Niche differentiation between diploid and hexaploid *Aster amellus*. *Oecologia* 158:463–472

Ramsey J, Schemske DW. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29:467–501

Ramsey J, Ramsey TS. 2014. Ecological studies of polyploidy in the 100 years following its discovery. *Philosophical Transactions of the Royal Society B* 369:20130352

Rešetnik I, Frajman B, Schönswetter P. 2016. Heteroploid *Knautia drymeia* includes *K. gussonei* and cannot be separated into diagnosable subspecies. *American Journal of Botany* 103:1300–1313

Rešetnik P, Schönswetter M, Temunović M, *et al*. 2023. Diploid chastity vs. polyploid promiscuity – Extensive gene flow among polyploid cytotypes blurs genetic, morphological and taxonomic boundaries among Dinaric taxa of *Knautia* (Caprifoliaceae). *Perspectives in Plant Ecology, Evolution and Systematics* 59:125730

Rice A, Šmarda P, Novosolov M, *et al*. 2019. The global biogeography of polyploid plants. *Nature Ecology & Evolution* 3:265–273

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Rojas-Andrés BM, Padilla-García N, de Pedro M, *et al*. 2020. Environmental differences are correlated with the distribution pattern of cytotypes in *Veronica* subsection *Pentasepalae* at a broad scale, *Annals of Botany* 125:471–484

Šemberová K, Svitok M, Marhold K, *et al*. 2023. Morphological and environmental differentiation as prezygotic reproductive barriers between parapatric and allopatric *Campanula rotundifolia* agg. cytotypes. *Annals of Botany* 131:71–86

Shields GF, and Procunier WS. 2019. Sympatric speciation in the *Simulium arcticum* s.l. complex (Diptera: Simuliidae): the Rothfels model updated. *Ecology and Evolution* 9:8265–8278

Šmarda P, Hejcman M, Březinová A, *et al*. 2013. Effect of phosphorus availability on the selection of species with different ploidy levels and genome sizes in a long-term grassland fertilization experiment. *New Phytologist* 200:911–921

Suda J, Malcová R, Abazid D. *et al.* 2004. Cytotype distribution in *Empetrum* (Ericaceae) at various spatial scales in the Czech Republic. *Folia Geobotanica* 39:161–171

Treier UA, Broennimann O, Normand S, *et al.* 2009. Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa*. *Ecology* 90:1366–1377

Van de Peer Y, Mizrachi E, Marchal K. 2017. The evolutionary significance of polyploidy. *Nature Reviews Genetics* 18:411–424

Van de Peer Y, Ashman TL, Soltis PS, *et al*. 2021. Polyploidy: an evolutionary and ecological force in stressful times. *The Plant Cell* 33:11–26

Visger CJ, Germain-Aubrey CC, Patel M, *et al*. 2016. Niche divergence between diploid and autotetraploid *Tolmiea*. *American Journal of Botany* 103:1396–1406

Walczyk AM, Hersch-Green EI. 2019. Impacts of soil nitrogen and phosphorus levels on cytotype performance of the circumboreal herb *Chamerion angustifolium*: implications for polyploid establishment. *American Journal of Botany* 106:906–921

Wei T, Simko V. 2017. R package 'corrplot': visualisation of a correlation matrix. https://github.com/taiyun/corrplot

Wefferling KM, Castro S, Loureiro J, *et al.* 2017. Cytogeography of the subalpine marsh marigold polyploid complex (*Caltha leptosepala* s.l., Ranunculaceae). *American Journal of Botany* 104:271–285

Weiss-Schneeweiss H, Emadzade K, Jang TS, *et al*. 2013. Evolutionary consequences, constraints and potential of polyploidy in plants. *Cytogenetic and Genome Research* 140:137–150

Wos G, Mořkovská J, Bohutínská M, *et al*. 2019. Role of ploidy in colonization of alpine habitats in natural populations of *Arabidopsis arenosa*. *Annals of Botany* 124:255–268

World Wildlife Fund. *Montane grasslands and shrublands* https://www.worldwildlife.org/biomes/montane-grasslands-and-shrublands

Wu J, Wang M, Zhu Z, *et al*. 2022. Cytogeography of the East Asian Tulips (*Amana*, Liliaceae). *Taxonomy* 2:145–159

Zhong J, Yi S, Ma L, *et al*. 2019. Evolution and phylogeography analysis of diploid and polyploid *Misgurnus anguillicaudatus* populations across China. *Proceedings of the Royal Society B* 286:20190076

Zozomová-Lihová J, Malánová-Krásná I, Vít P, *et al*. 2015. Cytotype distribution patterns, ecological differentiation, and genetic structure in a diploid-tetraploid contact zone of *Cardamine amara*. *American Journal of Botany* 102:1380–1395

Table 2.1 List of species/genus names and geographic regions of studies that were used in the meta-analysis. Where possible, we present a summary of reported ploidy level environmental distribution based on elevation and environmental variables. A dash represents an instance where ploidy level distribution based on elevation and environmental variables was not explicitly stated in the study. Overall, the studies represented a total of 70 species. N = total number of populations. The number of diploid populations is presented in parentheses.

Species/Genus, ploidy level range, life history	Region	N (2*x*)	Ploidy level occurrences	Study
Cardamine amara, diploids and tetraploids, perennial	North of the Alps	302 (108)	Polyploids occupied high altitudes. Likelihood of polyploid distribution decreased with increasing temperature	Zozomová-Lihová *et al*. (2015)
Arabidopsis arenosa, diploids and tetraploids, biennial or short-lived perennial	Western Carpathians	45 (24)	Environmental conditions did not differ between diploid and polyploid populations	Wos *et al*. (2019)

Oxalis obtusa, diploids to octoploids, perennial	Greater Cape Floristic Region	355 (84)	Vegetation type largely influenced polyploid distribution. Precipitation parameters were associated with ploidy level distribution	Krejčíková *et al*. (2013)
Arabidopsis arenosa, diploids and tetraploids, biennial or short-lived perennial	Europe	194 (88)	No evidence for habitat differentiation between ploidy levels	Kolář *et al*. (2016)
Festuca amethystina, diploids and tetraploids, perennial	Central and South-Eastern Europe	71 (24)	Diploids tended to occur at high altitudes. Mean temperature of the driest quarter was the most influential predictor for polyploid distribution	Kiedrzyński *et al*. (2021)
Alyssum repens, diploids and tetraploids, annual or perennial	Southeastern Alps and the Carpathians	19 (8)	–	Melichárková *et al.* (2019)

Campanula rotundifolia, diploids to hexaploids, perennial	Central Europe	231 (123)	Evidence for niche overlap between diploids and polyploids. Diploids had a broader environmental niche than polyploids	Šemberová *et al*. (2023)
Thymus section *Mastichina,* diploids and tetraploids, perennial	Iberian Peninsula	29 (20)	Weak correlation of geographical variables with ploidy level distribution	Girón *et al*. (2012)
Lycoris radiata, diploids to tetraploids, perennial	East Asia	114 (52)	Broad distribution range for triploids than diploids	Liu *et al*. (2019)
Oxalis obtusa, diploids to octoploids, perennial	Greater Cape Floristic Region	112 (39)	No evidence for strong ecological preferences for diploids and tetraploids. Diploids occurred at lower altitudes than polyploids	Krejčíková *et al*. (2013)
Artemisia sieberi, diploids and tetraploids, perennial	Iran	34 (9)	No relationship between ploidy level and distribution by latitude and altitude	Jalili *et al*. (2013)

Potentilla rigoana, diploids to heptaploids, perennial	Apennines	21 (8)	–	Dobeš *et al*. (2013)
Amana, diploids to tetraploids, perennial	Sino-Japanese Floristic Subregion	94 (67)	Diploids reached higher latitudes than polyploids	Wu *et al*. (2022)
Seseli libanotis, diploids and tetraploids, biennial or perennial	Central Europe and adjacent areas	147 (95)	No segregation by elevation between ploidy levels in Slovakia and Czechia	Němečková *et al*. (2018)
Centaurea maculosa, diploids and tetraploids, biennial or perennial	Europe	93 (53)	No prevalent shift in ploidy level distribution by geographical pattern	Treier *et al*. (2009)
Knautia, diploids to hexaploids, perennial	Western Balkan Peninsula	54 (21)	Diploids tended to occur in higher summer and winter temperatures than polyploids	Rešetnik *et al*. (2023)

Veronica subsection *Pentasepalae,* diploids to octoploids, perennial	Europe and North Africa	207 (103)	Polyploids tended to occur in wet and cold habitats compared to diploids	Rojas-Andrés *et al*. (2020)
Symphytum officinale, diploids and tetraploids, perennial	Europe	156 (38)	Niche overlap between ploidy levels	Kobrlová *et al*. (2022)
Knautia drymeia, diploids and tetraploids, perennial	Southeastern Europe and adjacent areas	57 (23)	–	Rešetnik *et al.* (2016)
Brachypodium distachyon, diploids and tetraploids, annual	Iberian Peninsula	57 (20)	Link between aridity and polyploid distribution	Manzaneda *et al*. (2012)
Turnera sidoides subsp. *pinnatifida,* diploids to hexaploids, perennial	Central Argentina	26 (7)	Diploids occurred at high altitudes, in wet and cold habitats, and polyploids had a broad ecological spectrum	Elías *et al*. (2011)

Heuchera cylindrica, diploids and tetraploids, perennial	Pacific Northwest of North America	43 (29)	Niche overlap between ploidy levels	Godsoe *et al*. (2013)
Parasenecio kamtschaticus, diploids and tetraploids, perennial	East Asia	30 (10)	Broad and high-elevation geographical distribution for polyploids	Kudo and Hirao (2020)
Chrysanthemum indicum, diploids to octoploids, perennial	Southeast Asia	47 (10)	–	Li *et al.* (2014)
Polylepis australis, diploids to hexaploids, perennial	Argentina	27 (8)	Environmental variables were not correlated to ploidy level distribution	Kessler *et al*. (2014)
Tolmiea, diploids and tetraploids, perennial	Pacific Northwest of North America	31 (13)	Niche differentiation found between ploidy levels	Visger *et al.* (2016)
Centaurea jacea, diploids and tetraploids, perennial	Belgian Ardennes	26 (9)	Similar habitat characteristics between ploidy levels	Hardy *et al*. (2000)

Empetrum, diploids to tetraploids, perennial	Czech Republic and one adjacent Polish locality	37 (20)	Habitat differentiation between diploids and polyploids	Suda *et al.* (2004)

Table 2.2 Correlation analyses of environmental variables at the highest and lowest elevations (i.e., correlation between each variable and either minimum or maximum elevations) where diploids and polyploids occurred across 28 studies. PREC = precipitation, T2M_RANGE = temperature range, RH2M = relative humidity, GWETPROF = profile soil moisture, UV_INDEX = ultraviolet index, and LW_DWN = downward thermal infrared irradiance. Extremely small significant *P* values are represented by 0.0001. A Spearman rank-correlation coefficient was used to test the correlation between the variables and the highest diploid and highest polyploid elevations as well as lowest diploid and lowest polyploid elevations across studies.

	High elevations		Low elevations	
Variable	rho	*P* value	rho	*P* value
LW_DWN	-0.14	0.0001	-0.11	0.0001
T2M_RANGE	0.31	0.0001	0.51	0.0001
UV_INDEX	0.20	0.0001	0.08	0.0001
GWETPROF	-0.35	0.0001	-0.21	0.0001
PREC	-0.12	0.0001	-0.10	0.0001
RH2M	-0.25	0.0001	-0.26	0.0001

Table 2.3 Summary of results from a generalized linear model of the effect of environmental variables on the presence of diploids and polyploids at high elevations across 28 studies. PREC = precipitation, T2M_RANGE = temperature range, RH2M = relative humidity, GWETPROF = profile soil moisture, UV_INDEX = ultraviolet index, LW_DWN = downward thermal infrared irradiance, with geographic region included as a covariate. Extremely small significant *P* values are represented by 0.0001. Odds ratio with 95% confidence intervals are presented. The relative importance (standardized, average contribution per variable) of environmental variables for the presence of polyploids at high elevations is also presented. The total variance explained by the model was 0.003. We used the packages *pscl* and *domir* to compute the overall fit of the model as well as the relative importance of each variable. The estimates are for polyploids compared to the diploid base across studies.

Variable	Estimate	SE	*z* value	*P*	Odds ratio	2.5 %	97.5 %	Standardized	Ranks
(Intercept)	-0.12	0.06	-1.97	0.05	0.89	0.79	1.00		
PREC	-0.003	0.001	-3.21	0.001	1.00	1.00	1.00	0.01	7
T2M_RANGE	0.02	0.002	8.28	0.0001	1.02	1.01	1.02	0.07	4
RH2M	0.005	0.0004	11.76	0.0001	1.005	1.00	1.01	0.12	3
GWETPROF	-0.43	0.04	-11.26	0.0001	0.65	0.60	0.70	0.28	2
UV_INDEX	-0.06	0.01	-6.26	0.0001	0.94	0.92	0.96	0.05	5
LW_DWN	0.001	0.0001	6.02	0.0001	1.001	1.001	1.00	0.02	6
Region	-0.06	0.004	-15.29	0.0001	0.94	0.93	0.95	0.46	1

Figure Legend

Fig. 2.1 Diploid and polyploid populations where daily environmental data were extracted for the current study. Sampled points represent the highest diploid and highest polyploid elevations per study.

Fig. 2.2 Effect sizes with 95% confidence intervals for polyploid distribution based on elevation across 28 studies. The dashed line represents the small (grey), moderate (black), and large (red) effect size benchmarks. Effect sizes were calculated as Hedge's *g*. Studies that produced large effect sizes are indicated in bold. Falling to the left or right of the effect size at = 0 indicates negative (<0) and positive (>0) effect sizes.

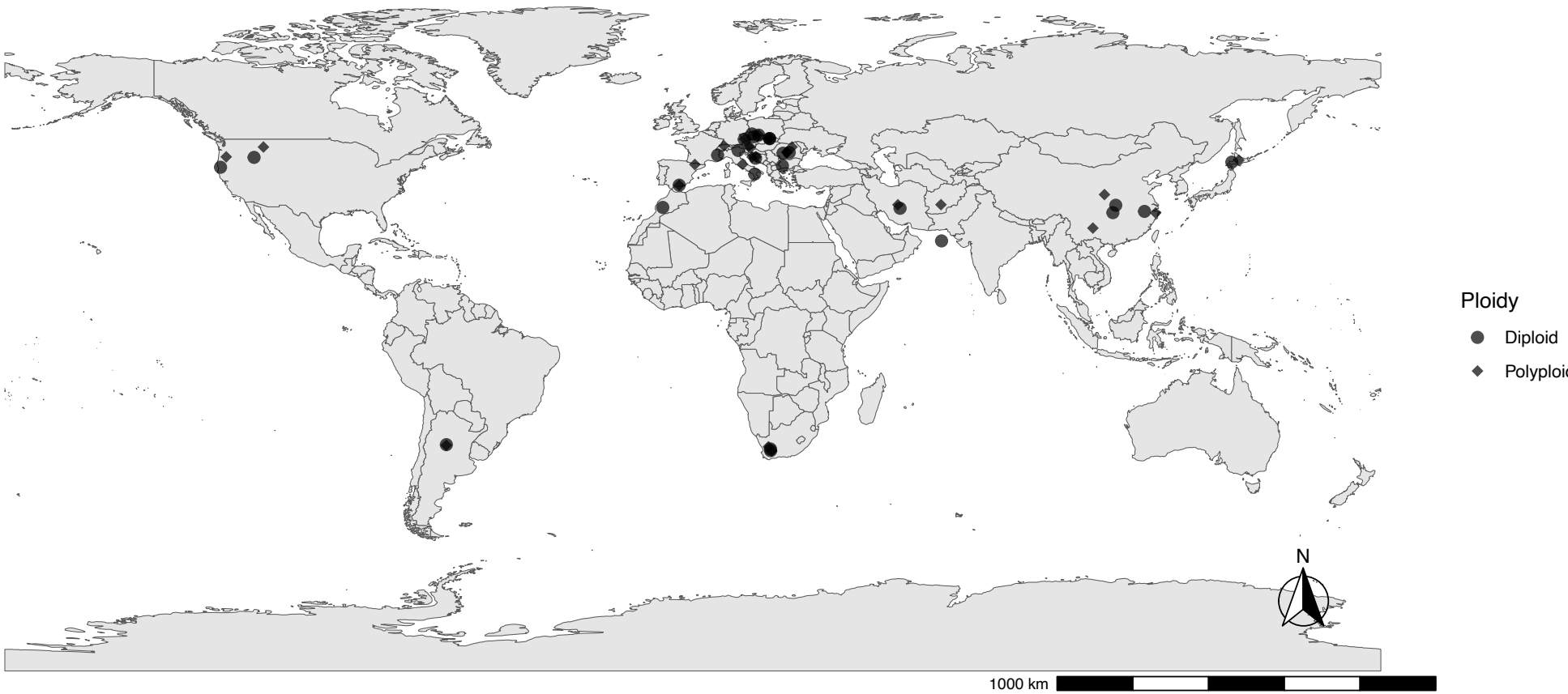
Fig. 2.3 Distribution of daily data of environmental variables between diploids and polyploids across 28 studies representing different plant systems and geographical regions. Using Kruskal-Wallis tests, we found that there were significant differences in the variables between diploids and polyploids at $P = 0.0001$ unless indicated otherwise by a P value > 0.05; the data comprised >180k points for 10 years of daily environmental data across studies. Ultraviolet (UV) index and downward thermal infrared irradiance did not significantly differ between diploids and polyploids and were omitted from subsequent comparisons among regions. 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 outliers which are represented by black dots.

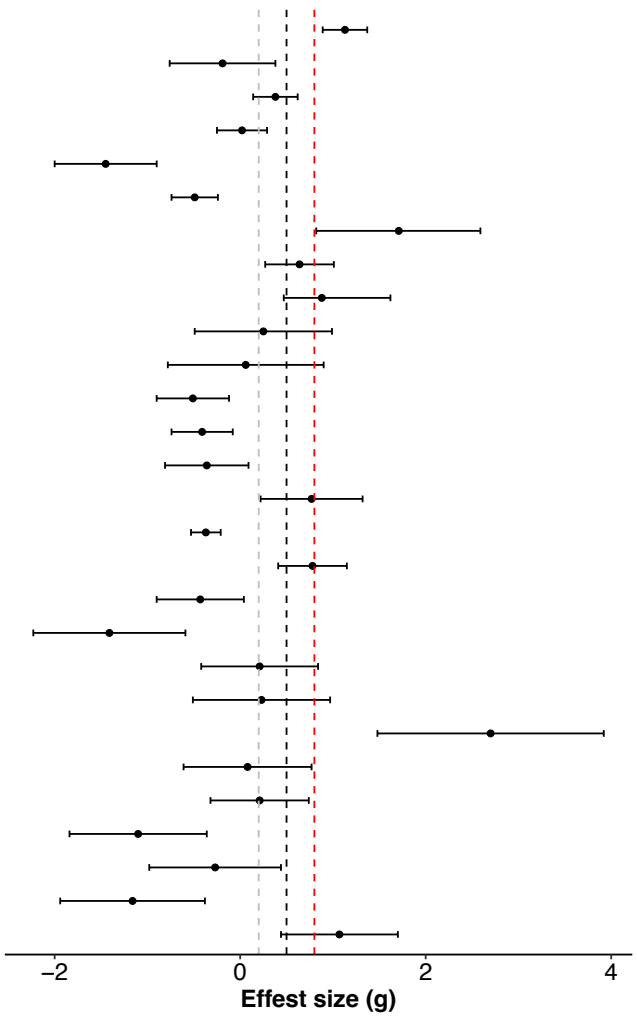
Fig. 2.4 Comparison of daily data of environmental variables between diploids (white) and polyploids (grey) for 28 studies across regions in Africa (AF), Europe (EU), Asia (AS), Europe and North Africa (EU & AF), North America (NA), and South America (SA) using Kruskal-Wallis tests. The variables were significantly different between diploids and polyploids at $P = 0.05$ unless indicated otherwise by a P value > 0.05. 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 outliers which are represented by black dots.

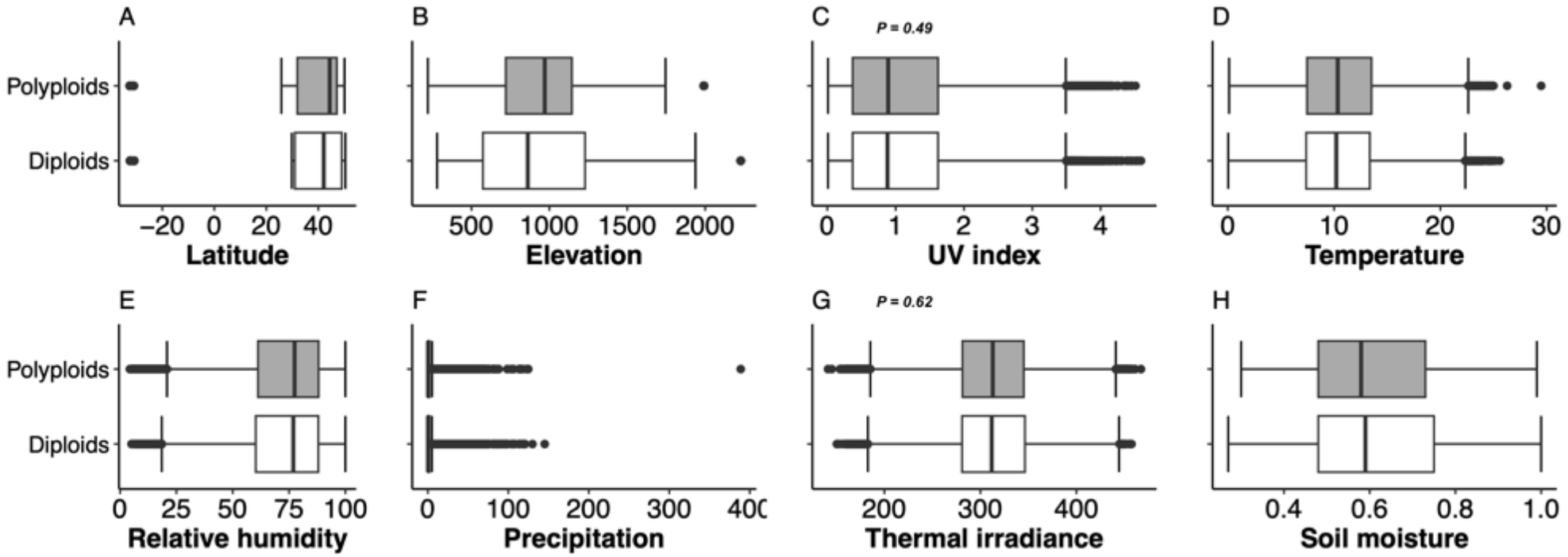
Fig. 2.5 Principal Component Analysis (PCA; PC = principal components) of six environmental variables that were assessed for possible relationships with the highest and lowest elevations where diploids and polyploids occurred across 28 studies. PREC = precipitation, T2M = temperature range, RH2M = relative humidity, PROF = profile soil moisture, UV_INDEX = ultraviolet index, and LW_DWN = downward thermal infrared irradiance. The PCA was constructed using the package *corrplot*. Each dot represents either the minimum or maximum elevation for all ploidy levels of a study.

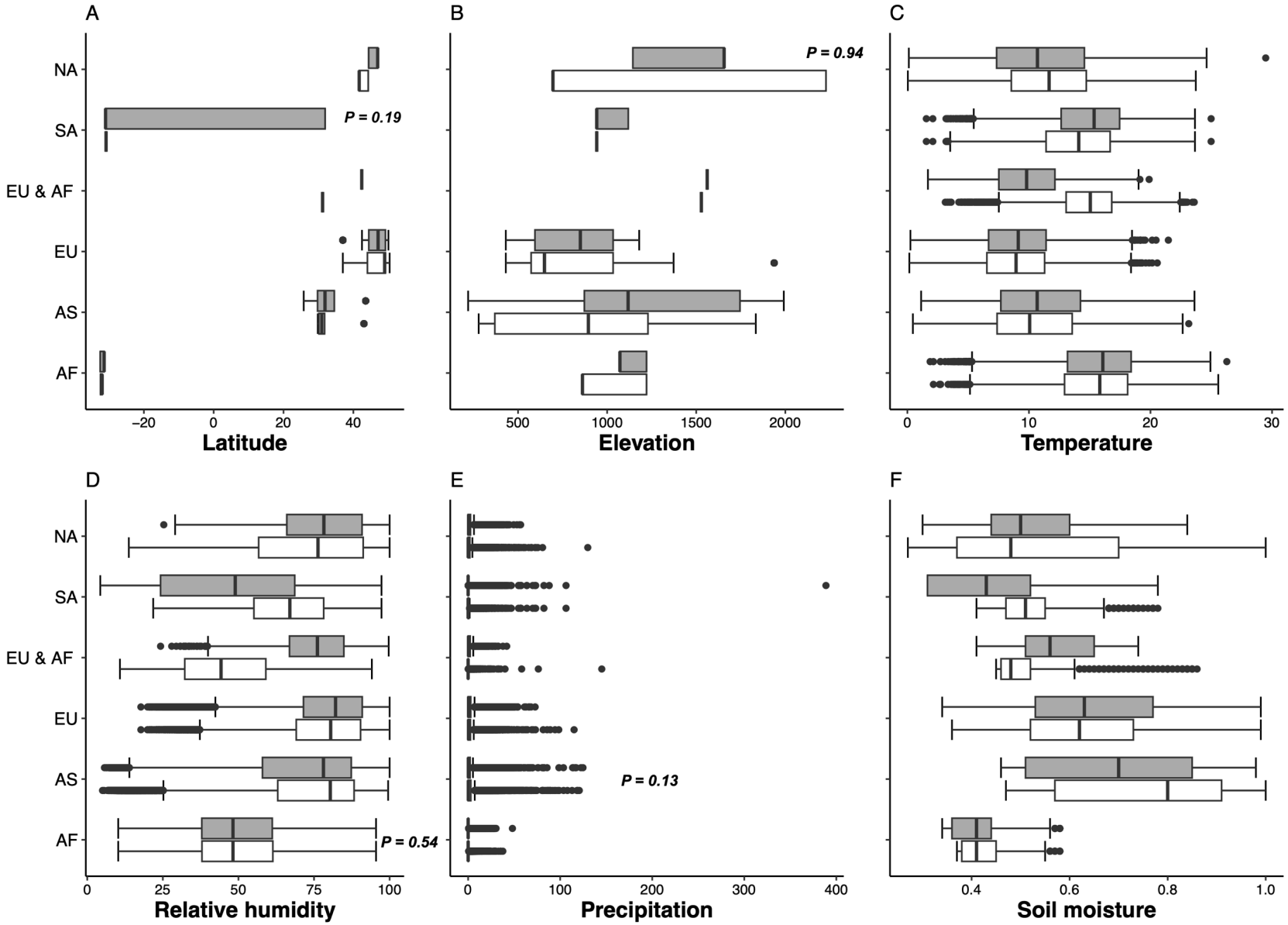
Fig. 2.6 Principal Component Analysis (PCA; PC = principal components) of six environmental variables that were assessed for possible relationships with the highest and lowest elevations where diploids and polyploids of *Cardamine amara* occurred. PREC = precipitation, T2M = temperature range, RH2M = relative humidity, PROF = profile soil moisture, UV_INDEX = ultraviolet index, and LW_DWN = downward thermal infrared irradiance. The PCA was constructed using the package *corrplot*.

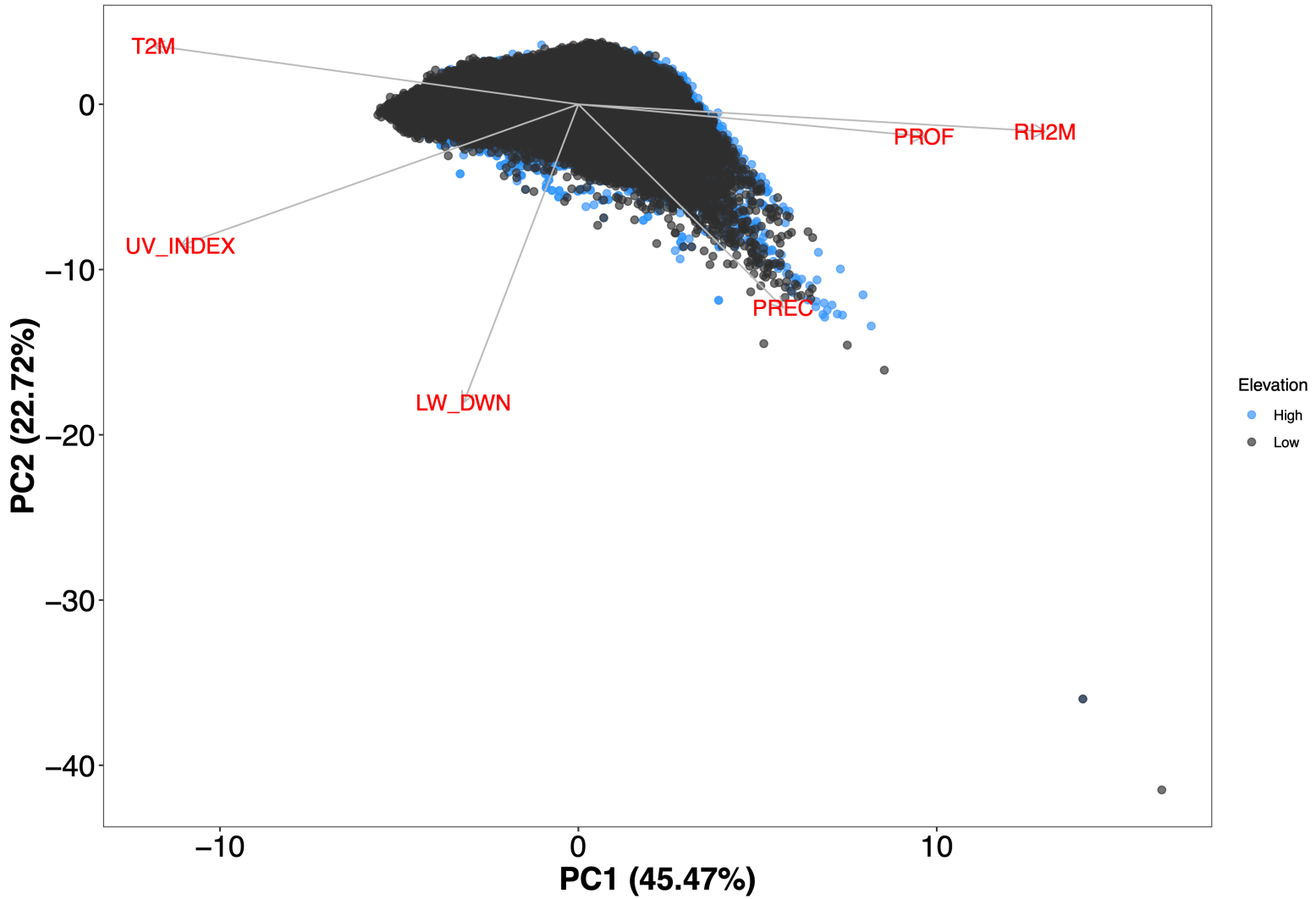

Ploidy
Diploid
Polyploid
N
1000 km

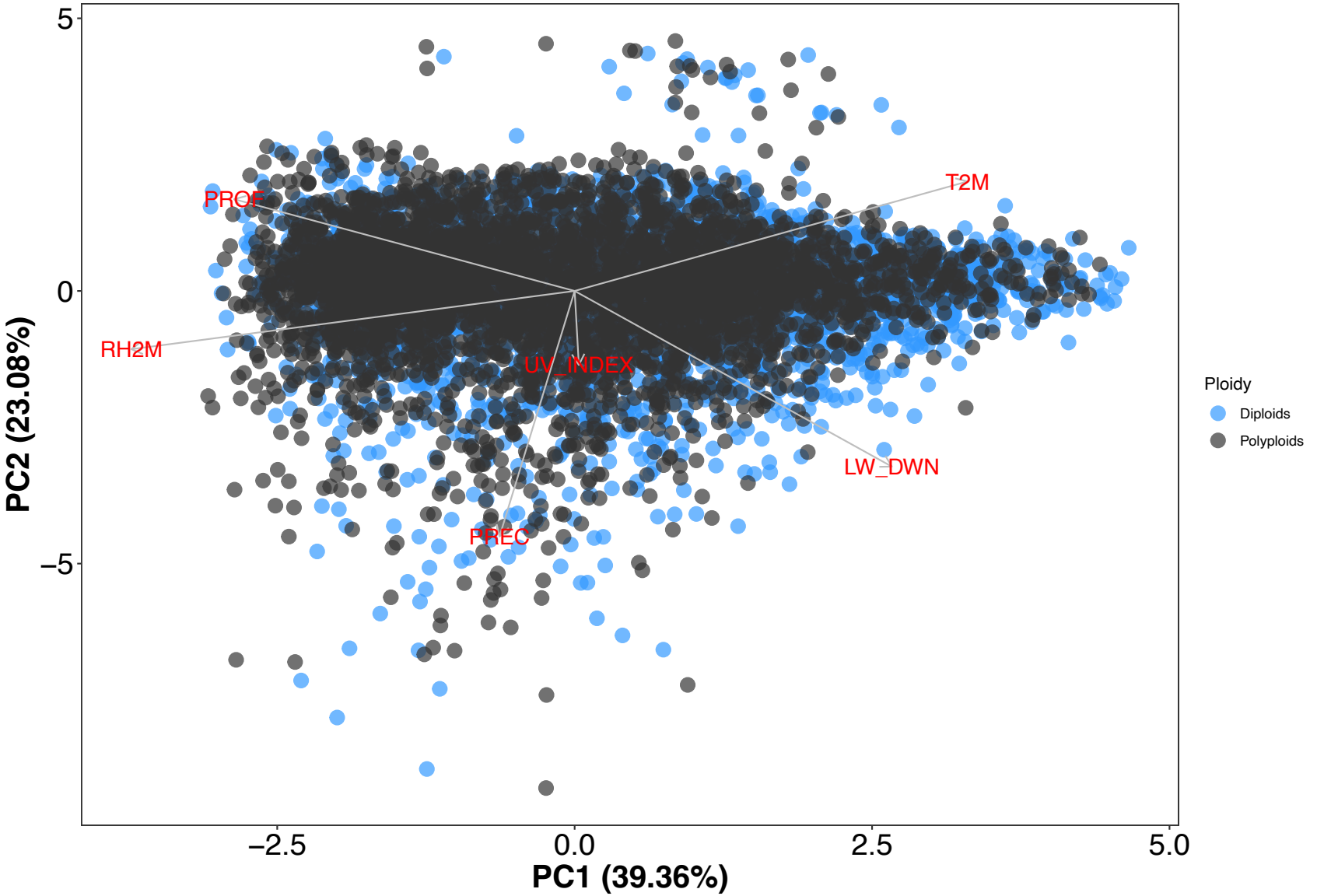


Zozomová-Lihová *et al*. (2015)	North of the Alps
Wos *et al*. (2019)	Western Carpathians
Krejčíková *et al*. (2013)	Greater Cape Floristic Region
Kolář *et al*. (2016)	Europe
Kiedrzyński *et al*. (2021)	Central and South-Eastern Europe
Šemberová *et al*. (2023)	Central Europe
Girón *et al*. (2012)	Iberian Peninsula
Liu *et al*. (2019)	East Asia
Krejčíková *et al*. (2013)	Greater Cape Floristic Region
Jalili *et al*. (2013)	Iran
Dobeš *et al*. (2013)	Apennines
Wu *et al*. (2022)	Sino-Japanese Floristic Subregion
Němečková *et al*. (2018)	Central Europe and adjacent areas
Treier *et al*. (2009)	Europe
Rešetnik *et al*. (2023)	Western Balkan Peninsula
Rojas-Andrés *et al*. (2020)	Europe and North Africa
Kobrlová *et al*. (2022)	Europe
Manzaneda *et al*. (2012)	Iberian Peninsula
Elías *et al*. (2011)	Central Argentina
Godsoe *et al*. (2013)	Pacific Northwest of North America
Kudo and Hirao (2020)	East Asia
Melichárková *et al.* (2019)	Southeastern Alps and the Carpathians
Li *et al.* (2014)	Southeast Asia
Rešetnik *et al.* (2016)	Southeastern Europe and adjacent areas
Kessler *et al*. (2014)	Argentina
Visger *et al.* (2016)	Pacific Northwest of North America
Hardy *et al*. (2000)	Belgian Ardennes
Suda *et al.* (2004)	Czech Republic and one adjacent Polish locality

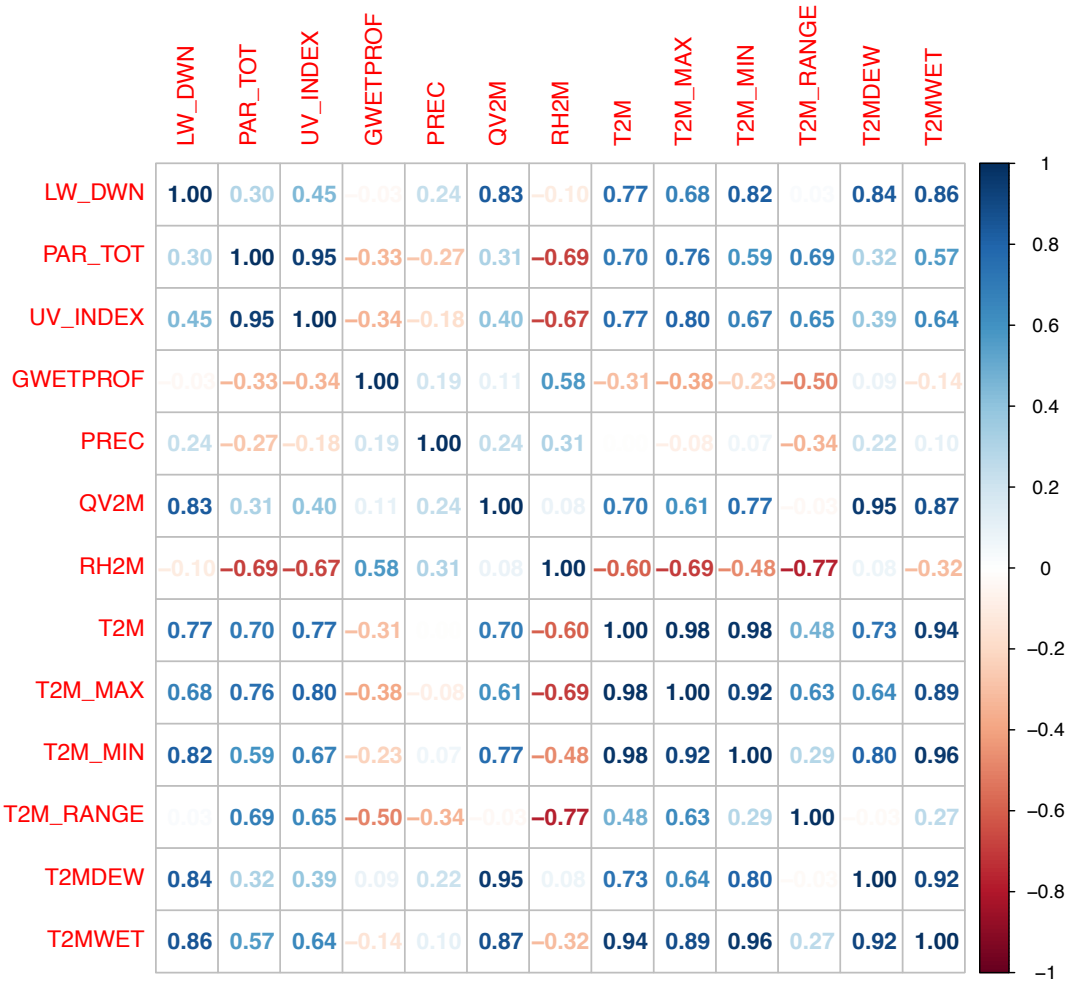

A
Polyploids
Diploids
−20 0 20 40
Latitude
B
500 1000 1500 2000
Elevation
C
P = 0.49
0 1 2 3 4
UV index
D
0 10 20 30
Temperature
E
Polyploids
Diploids
0 25 50 75 100
Relative humidity
F
0 100 200 300 400
Precipitation
G
P = 0.62
200 300 400
Thermal irradiance
H
0.4 0.6 0.8 1.0
Soil moisture


A
B
C
D
E
F
NA
SA
EU & AF
EU
AS
AF
P = 0.19
P = 0.94
P = 0.54
P = 0.13
−20
0
20
40
Latitude
500
1000
1500
2000
Elevation
0
10
20
30
Temperature
0
25
50
75
100
Relative humidity
0
100
200
300
400
Precipitation
0.4
0.6
0.8
1.0
Soil moisture


T2M
PROF
RH2M
UV_INDEX
PREC
LW_DWN
0
−10
−20
−30
−40
PC2 (22.72%)
−10
0
10
PC1 (45.47%)
Elevation
High
Low


5
0
−5
−2.5
0.0
2.5
5.0
PC2 (23.08%)
PC1 (39.36%)
PROF
T2M
RH2M
UV_INDEX
LW_DWN
PREC
Ploidy
Diploids
Polyploids

SUPPLEMENTARY INFORMATION



	LW_DWN	PAR_TOT	UV_INDEX	GWETPROF	PREC	QV2M	RH2M	T2M	T2M_MAX	T2M_MIN	T2M_RANGE	T2MDEW	T2MWET
LW_DWN	1.00	0.30	0.45	−0.03	0.24	0.83	−0.10	0.77	0.68	0.82	−0.03	0.84	0.86
PAR_TOT	0.30	1.00	0.95	−0.33	−0.27	0.31	−0.69	0.70	0.76	0.59	0.69	0.32	0.57
UV_INDEX	0.45	0.95	1.00	−0.34	−0.18	0.40	−0.67	0.77	0.80	0.67	0.65	0.39	0.64
GWETPROF	−0.03	−0.33	−0.34	1.00	0.19	0.11	0.58	−0.31	−0.38	−0.23	−0.50	0.09	−0.14
PREC	0.24	−0.27	−0.18	0.19	1.00	0.24	0.31	−0.30	−0.08	0.07	−0.34	0.22	0.10
QV2M	0.83	0.31	0.40	0.11	0.24	1.00	0.08	0.70	0.61	0.77	−0.03	0.95	0.87
RH2M	−0.10	−0.69	−0.67	0.58	0.31	0.08	1.00	−0.60	−0.69	−0.48	−0.77	0.08	−0.32
T2M	0.77	0.70	0.77	−0.31	−0.30	0.70	−0.60	1.00	0.98	0.98	0.48	0.73	0.94
T2M_MAX	0.68	0.76	0.80	−0.38	−0.08	0.61	−0.69	0.98	1.00	0.92	0.63	0.64	0.89
T2M_MIN	0.82	0.59	0.67	−0.23	0.07	0.77	−0.48	0.98	0.92	1.00	0.29	0.80	0.96
T2M_RANGE	−0.03	0.69	0.65	−0.50	−0.34	−0.03	−0.77	0.48	0.63	0.29	1.00	−0.03	0.27
T2MDEW	0.84	0.32	0.39	0.09	0.22	0.95	0.08	0.73	0.64	0.80	−0.03	1.00	0.92
T2MWET	0.86	0.57	0.64	−0.14	0.10	0.87	−0.32	0.94	0.89	0.96	0.27	0.92	1.00

Fig. S1 Correlation matrix for 13 environmental variables that were assessed for possible relationships with the presence of polyploids at high elevations across 28 studies. Six variables were selected using a correlation coefficient not exceeding 0.70 for subsequent analyses: PREC = precipitation, T2M_RANGE = temperature range, RH2M = relative humidity, GWETPROF = profile soil moisture, UV_INDEX = ultraviolet index, and LW_DWN = downward thermal infrared irradiance. T2MWET = Wet bulb temperature at 2 meters (ºC), T2MDEW = dew/frost point at 2 meters (ºC), T2M = temperature at 2 meters (ºC), T2M_MIN = temperature at 2 meters minimum (ºC), T2M_MAX = temperature at 2 meters maximum (ºC), QV2M = specific humidity at 2 meters (g/kg), and PAR-TOT = all sky surface PAR total (W/m^2).

Table S1 Summary of Principal Component Analysis results of six environmental variables that were assessed for possible relationships with the highest and lowest elevations where diploids and polyploids occurred across 28 studies. PREC = precipitation, T2M_RANGE = temperature range, RH2M = relative humidity, GWETPROF = profile soil moisture, UV_INDEX = ultraviolet index, and LW_DWN = downward thermal infrared irradiance.

	PC1	PC2
Factor loadings		
PROF	0.40	-0.08
LW_DWN	-0.13	-0.76
PREC	0.24	-0.52
RH2M	0.55	-0.07
T2M	-0.50	0.15
UV_INDEX	-0.47	-0.36
Proportion of Variance	0.45	0.23
Eigenvalue	2.73	1.36

CHAPTER 3

MORPHOLOGICAL AND HABITAT DIFFERENTIATION BETWEEN DIPLOIDS AND TETRAPLOIDS OF A DRAKENSBERG NEAR-ENDEMIC TAXON, *RHODOHYPOXIS BAURII* VAR. *PLATYPETALA* (HYPOXIDACEAE)

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Published in *Austral Ecology* as:

Mtileni MP, Oberlander KC, and Glennon KL. 2024. Morphological and habitat differentiation between diploids and tetraploids of a Drakensberg near-endemic taxon, *Rhodohypoxis baurii* var. *platypetala* (Hypoxidaceae). *Austral Ecology* 49:e13502. https://doi.org/10.1111/aec.13502

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? and 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.

Key words: 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; Ramsey and Schemske, 1998; Otto and Whitton, 2000), is widely considered an important mechanism of evolution in angiosperms (Wood *et al*., 2009; Weiss-Schneeweiss *et al*., 2013; Ramsey and Ramsey, 2014). Polyploid plants are globally distributed and environmental factors such as annual mean temperature and latitude have been suggested as key drivers of this global distribution (Rice *et al*., 2019, Bureš *et al*., 2024). 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 *Dicerothamnus 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., Husband and Schemske, 1998; Castro *et al*., 2018, 2019; Afonso *et al*., 2021), may be governed by the putative competitive advantage polyploids have over diploids in stressful environmental conditions like drought, heat stress and salinity (Maherali *et al*., 2009; Zhang *et al*., 2010; Liu *et al*., 2011; Chao *et al*., 2013; Yang *et al*., 2014; Godfree *et al*., 2017; but see Wei *et al*., 2020; Mtileni *et al*., 2021). 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 (Leitch and Leitch, 2008; Faizullah *et al*., 2021). 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 (Glennon *et al*., 2014; Castro *et al*., 2019). 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 Krejčíková *et al*., 2013; Veselý *et al*., 2020; Elliot *et al*., 2023, Chumová *et al*., 2023, 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 and Burtt, 1978). The genus-level taxonomy is contentious, so we are following the taxonomic designation of Hilliard and Burtt (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 and Burtt, 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 Burtt (1978). Leaf sample collections for flow cytometry were carried out in the peak growing season for *R. baurii* var. *platypetala* (November) over two 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 one-meter squared quadrat in each population six times and sampling five individuals inside the quadrat (one from each corner and one in the center). Quadrats were positioned at least 3m 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 3m 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 labeled 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 3.1). 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 3.1). Generalized linear models were constructed using the base *stats* package (R version 4.2.1; R 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 climactic 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 3.1). 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 cm^2 each) were chopped together in 1000 µl of Otto I (0.1M citric acid monohydrate, 0.5 ml of Tween 20 and 100 ml of dH_2O) using a new razor blade. The homogenate was filtered into a sample tube through a 30 µm mesh (Sysmex Partec GmbH, Görlitz, Germany) and then incubated for approximately 20 minutes at room temperature. Then 1000 µl of Otto II buffer (0.72g of $NA_2HPO_4.H_20$, 10 ml of dH_2O, 10µl of DAPI and 20 µl of beta-Mercapto-ethanol) was added and the solution was incubated for an additional 10 minutes 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 analyzed 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 meters above the surface of the Earth (°C), 2) dew/frost point at 2 meters above the surface of the Earth (°C), 3) wet bulb temperature at 2 meters above the surface of the Earth (°C), 4) Earth skin temperature (°C), 5) maximum temperature at 2 meters above the surface of the Earth (°C), 6) minimum temperature at 2 meters above the surface of the Earth (°C), 7) all sky surface longwave downward irradiance (horizontal infrared radiation intensity from sky; W m^{-2}), 8) specific humidity at 2 meters above the surface of the Earth (g kg^{-1}), 9) relative humidity at 2 meters above the surface of the Earth (%), 10) precipitation corrected (mm/day), 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 and Simko, 2017) to reduce the number of correlated climactic 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 meters above the surface of the Earth (°C), all sky surface longwave downward irradiance (horizontal infrared radiation intensity from sky; W m^{-2}), precipitation corrected (mm/day), and profile soil moisture (0–1). We also included possible interactions between these variables (see Table 3.4 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 3.1 and 3.5) per our collecting permit and landowner permission for analyses of available nitrogen (%; analyzed as NH_4-N), available potassium (mg kg^{-1}) and phosphorus (mg kg^{-1}) contents (analyzed 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 (200g 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 (Fig. 3.1B), triploids (Fig. 3.1C), and tetraploids (Fig. 3.1D; Appendix S1, S2), after which four tetraploid populations, 12 diploid populations, and one mixed-ploidy population were identified (Table 3.1; Fig. 3.2). The mixed-ploidy population was predominantly diploid, with only seven of the 34 sampled individuals found to be triploids and no tetraploids were found.

Geographic distribution of ploidy levels

Diploids occurred between 1460 m a.s.l. and 2060 m a.s.l., whereas tetraploids occurred between 1240 m a.s.l. and 1750 m a.s.l. (Table 3.1). While diploids reached the highest recorded altitudes for the current study than tetraploids (Table 3.1; Fig. 3.2), 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 3.1). 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 3.2), and diploids possessed more leaves than tetraploids (Fig. 3.3). The number of flowers and buds, and largest-leaf length, were not significantly affected by ploidy (Table 3.2). Tetraploids possessed broader leaves and larger flowers and larger plants than diploids (Fig. 3.3, 3.4), but both ploidy and population had a significant effect on these traits (Table 3.2). 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 3.3). Tetraploids generally occurred in warmer and more humid conditions with increased infrared radiation intensity than diploids (Table 3.3). Tetraploid populations also experienced higher daily mean precipitation than diploid populations (diploid pop mean = 2.83 mm/day, tetraploid pop mean = 3.14 mm/day; 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 3.3). The mixed-ploidy and 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 climactic 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 were some overlap between diploid and tetraploid populations (Appendix S6, 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 3.4).

Soil pH was similar among the four analyzed populations (pH = 4.1 – 4.3; Table 3.5). 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 3.5).

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., Hardy *et al*., 2000; Afonso *et al*., 2021). 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 were 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 has also been observed in both the southern and northern hemispheres. In *Chamerion 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 (Leitch and Leitch, 2008; Faizullah *et al*., 2021). 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 (Walczyk and Hersch-Green, 2019; Anneberg and Segraves, 2020). In *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 and 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 and Segraves, 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 and 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 and 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.

ACKNOWLEDGMENTS

We thank B. Ferreira, T. Moloi, and H. Niemann for their assistance with fieldwork. We also thank J. Braby, P. Mackenzie, and E. Smith for helping us find additional populations. We thank S. Ntshangase for her assistance with flow cytometric analyses. We thank two anonymous reviewers for their feedback which improved this paper. Collections were made under permits from eKZN (OP 2961/2021) and South African National Parks (SS591) to M.P.M. K.L.G and M.P.M designed the study. M.P.M performed data collection and statistical analyses and prepared the first draft. K.L.G provided input on subsequent drafts. K.C.O provided input for flow cytometric analyses and input on the final draft. All authors approved the final manuscript.

REFERENCES

Anneberg TJ, Segraves KA. 2020. Nutrient enrichment and neopolyploidy interact to increase lifetime fitness of *Arabidopsis thaliana*. *Plant and Soil* 456: 439–453.

Afonso A, Loureiro J, Arroyo J, Olmedo-Vicente E, Castro S. 2021. Cytogenetic diversity in the polyploid complex *Linum suffruticosum* s.l. (Linaceae). *Botanical Journal of the Linnean Society* 195: 216–232.

Bureš P, Elliott TL, Veselý P, *et al*. 2024. The global biogeography of angiosperm genome size is shaped by climate and range size. *New Phytologist*. https://doi.org/10.1111/nph.19544.

Castro M, Castro S, Figueiredo A, Husband BC, Loureiro J. 2018. Complex cytogeographical patterns reveal a dynamic tetraploid-octoploid contact zone. *AoB PLANTS* 10: 1–18.

Castro M, Loureiro J, Serrano M, *et al*. 2019. Mosaic distribution of cytotypes in a mixed-ploidy plant species, *Jasione montana:* nested environmental niches but low geographic overlap. *Botanical Journal of the Linnean Society* 190: 51–66.

Chao DY, Dilkes B, Luo H, *et al*. 2013. Polyploids exhibit higher potassium uptake and salinity tolerance in *Arabidopsis*. *Science* 341: 658–659.

Chumová Z, Monier Z, Šemberova K, *et al*. 2023. Diploid and tetraploid cytotypes of the flagship Cape species *Dicerothamnus rhinocerotis* (Asteraceae): variation in distribution, ecological niche, morphology and genetics. *Annals of Botany* mcad084. https://doi.org/10.1093/aob/mcad084

de Wet J. 1971. Polyploidy and evolution in plants. *Taxon* 20: 29–35.

Doležel J, Greilhuber J, Suda J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols* 2: 2233–2244.

Elliot TL, Muasya AM, Bureš P. 2023. Complex patterns of ploidy in a holocentric plant clade (*Schoenus*, Cyperaceae) in the Cape biodiversity hotspot. *Annals of Botany* 131: 143–156.

Elías G, Sartor M, Neffa VGS. 2011. Patterns of cytotype variation of *Turnera sidoides* subsp. *pinnatifida* (Turneraceae) in mountain ranges of central Argentina. *Journal of Plant Research* 124: 25–34.

Faizullah L, Morton JA, Hersch-Green EI, Walczyk AM, Leitch AR, Leitch IJ. 2021. Exploring environmental selection on genome size in angiosperms. *Trends in Plant Science* 26: 1039–1049.

Glennon KL, Ritchie ME, Segraves KA. 2014. Evidence for shared broad-scale climatic niches of diploid and polyploid plants. *Ecology Letters* 17: 574–582.

Godfree RC, Marshall DJ, Young AG, Miller CH, Matthews S. 2017. Empirical evidence of fixed and homeostatic patterns of polyploid advantage in a keystone grass exposed to drought and heat stress. *Royal Society Open Science* 4: 170934.

Grab SW. 2013. Fine-Scale variations of near-surface-temperature lapse rates in the high Drakensberg Escarpment, South Africa: Environmental Implications. *Arctic, Antarctic, and Alpine Research* 45:500–514.

Hardy OJ, Vanderhoeven S, De Loose M, Meerts P. 2000. Ecological, morphological, and allozymic differentiation between diploid and tetraploid knapweeds (*Centaurea jacea*) from a contact zone in the Belgian Ardennes. *New Phytologist* 146: 281–290.

Hilliard OM, Burtt BT. 1978. Notes on some plants from southern Africa chiefly from Natal. VII. *Notes from the Royal Botanical Garden, Edinburgh* 36: 43–76.

Holm S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* 6: 65–70

Husband BC, Baldwin SJ, Suda J. 2013. The incidence of polyploidy in natural plant populations: Major patterns and evolutionary processes. In Leitch IJ, *et al*. ed: *Plant Genome Diversity Volume 2: physical structure, behaviour and evolution of plant genomes* Vienna: Springer-Verlag 255–276.

Husband BC, Schemske DW. 1998. Cytotype distribution at a diploid-tetraploid contact in *Chamerion* (*Epilobium*) *angustifolium* (Onagraceae). *American Journal of Botany* 85:1688–1694.

Kiedrzyński M. Zielińska KM, Jedrzejczyk I, *et al*. 2021. Tetraploids expanded beyond the mountain niche of their diploid ancestors in the mixed-ploidy grass *Festuca amethystina* L. *Scientific Reports* 11: 18735.

Krejčíková J, Sudová R, Oberlander K, Dreyer L, Suda J. 2013. The spatio-ecological segregation of different cytotypes of *Oxalis obtusa* (Oxalidaceae) in contact zones. *South African Journal of Botany* 88:62–68

Leitch AR, Leitch IJ. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* 320: 481–483.

Liu S, Chen S, Chen Y, Guan S, Yin D, Chen F. 2011. In vitro induced tetraploid of *Dendranthema nankingense* (Nakai) Tzvel. shows an improved level of abiotic stress tolerance. *Scientia Horticulturae* 127: 411–419.

Maherali H, Walden AE, Husband BC. 2009. Genome duplication and the evolution of physiological responses to water stress. *New Phytologist* 184: 721–731.

Mtileni MP, Venter N, Glennon KL. 2021. Ploidy differences affect leaf functional traits, but not water stress responses in a mountain endemic plant population. *South African Journal of Botany* 138: 76–83.

Otto SP, Whitton J. 2000. Polyploid incidence and evolution. *Annual Review of Genetics* 34: 401–437.

Porder S, Ramachandran S. 2013. The phosphorus concentration of common rocks – a potential driver of ecosystem P status. *Plant and Soil* 367: 41–55.

Ramsey J, Ramsey TS. 2014. Ecological studies of polyploidy in the 100 years following its discovery. *Philosophical Transactions of the Royal Society B* 369: 20130352.

Ramsey J, Schemske DW. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29: 467–501.

Rice A, Šmarda P, Novosolov M, *et al*. 2019. The global biogeography of polyploid plants. *Nature Ecology & Evolution* 3: 265–273.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Saito K. 1975. Studies on the occurrence of polyploidy and its contributions to the flower plant breeding. XII. On the nature and sterility in the spontaneous triploid cultivars of *Rhodohypoxis baurii* Nel. *Japanese Journal of Plant Breeding* 25: 355–362.

Schönswetter P, Lachmayer M, Lettner C, *et al*. 2007. Sympatric diploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps are separated along an altitudinal gradient. *Journal of Plant Research* 120: 721–725.

Thompson KA, Husband BC, Maherali H. 2014. Climatic niche differences between diploid and tetraploid cytotypes of *Chamerion angustifolium* (Onagraceae). *American Journal of Botany* 101: 1868 –1875.

Vaio M, Gardner A, Speranza P, Emshwiller E, Guerra M. 2014. Phylogenetic and cytogenetic relationships among species of *Oxalis* section *Articulatae* (Oxalidaceae). *Plant Systematics and Evolution* 302: 1253 – 1265.

Van der Peer Y, Mizrachi E, Marchal K. 2017. The evolutionary significance of polyploidy. *Nature Reviews Genetics* 18: 411–424.

Veselý P, Šmarda P, Bureš P, *et al*. 2020. Environmental pressures on stomatal size may drive plant genome size evolution: evidence from a natural experiment with Cape geophytes. *Annals of Botany* 126: 323–330.

Visger CJ, Germain-Aubrey CC, Patel M, Sessa EB, Soltis PS, Soltis DE. 2016. Niche divergence between diploid and autotetraploid *Tolmiea*. *American Journal of Botany* 103: 1396 –1406.

Walczyk AM, Hersch-Green EI. 2019. Impacts of soil nitrogen and phosphorus levels on cytotype performance of the circumboreal herb *Chamerion angustifolium*: implications for polyploid establishment. *American Journal of Botany* 106: 901–921.

Weiss-Schneeweiss H, Emadzade K, Jang TS, Schneeweiss GM. 2013. Evolutionary consequences, constraints, and potential of polyploidy in plants. *Cytogenic and Genome Research* 140: 137 –150.

Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH. 2009. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences of the United States of America* 106: 13875–13879.

Wei N, Du Z, Liston A, Ashman TL. 2020. Genome duplication effects on functional traits and fitness are genetic context and species dependent: studies of synthetic polyploid *Fragaria*. *American Journal of Botany* 107: 262–272.

Wei T, Simko V. 2017. R package 'corrplot': Visualisation of a Correlation Matrix. https://github.com/taiyun/corrplot

Yang C, Zhao L, Zhang H, *et al*. 2014. Evolution of physiological responses to salt stress in hexaploid wheat. *Proceedings of the National Academy of Sciences of the United States of America* 111: 11882–11887.

Zozomová-Lihová J, Malánová-Krásná I, Vít P, *et al*. 2015. Cytotype distribution patterns, ecological differentiation, and genetic structure in a diploid-tetraploid contact zone of *Cardamine amara*. *American Journal of Botany* 102:1380–1395.

Zhang XY, Hu CG, Yao JL. 2010. Tetraploidization of diploid *Dioscorea* results in activation of the antioxidant defense system and increased heat tolerance. *Journal of Plant Physiology* 167: 88–94.

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Appendix S1: Summary of flow cytometric results of sampled individuals.

Appendix S2: Summary results of sample/standard fluorescence ratios are shown for each ploidy level.

Appendix S3: Summary of correlation results between traits and climactic variables.

Appendix S4: Mean ± SE of 13 climactic variables compared among diploid, mixed, and tetraploid populations.

Appendix S5: Correlation matrix of climatic values assigned for the sampled populations.

Appendix S6: PCAs of sampled populations according to ploidy level and climatic variables.

Appendix S7: Summary of PCA results of four climatic variables.

Tables

Table 3.1 Ploidy level and altitude (m a.s.l.) of *Rhodohypoxis baurii* var. *platypetala* populations that were sampled for this study. (n) represents the number of individuals that were analyzed 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 Fig. 3.2 and Table 3.5.

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

Table 3.2 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*. Significant results are bolded, where 0.0001 represents *P* values that are extremely small to report.

(a) Number of leaves					(b) Number of flowers and buds			
Source	Estimate	SE	*t* value	*P*	Estimate	SE	*t* value	*P*
(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			
Source	Estimate	SE	*t* value	*P*	Estimate	SE	*t* value	*P*
(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			
Source	Estimate	SE	*t* value	*P*	Estimate	SE	*t* value	*P*

(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

Source	Estimate	SE	*t* value	*P*
(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**

Table 3.3 Mean, minimum (min.), and maximum (max.) values ± SE of 13 climactic variables compared between diploid (2*x*) and tetraploid (4*x*) populations of *Rhodohypoxis baurii* var. *platypetala*. Different letters within rows of each mean variable denote significant differences at $P = 0.0001$ between the populations.

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

Surface soil wetness (0–1)	0.28 ± 0.01	0.29 ± 0.03	0.60 ± 0.01 [a]	0.58 ± 0.02 [b]	0.94 ± 0.01	0.91 ± 0.01
Root zone soil wetness (0–1)	0.51 ± 0.01	0.48 ± 0.02	0.62 ± 0.01 [a]	0.58 ± 0.02 [b]	0.90 ± 0.02	0.81 ± 0.04
Profile soil moisture (0–1)	0.51 ± 0.01	0.49 ± 0.02	0.62 ± 0.01 [a]	0.59 ± 0.02 [b]	0.87 ± 0.03	0.80 ± 0.05

Table 3.4 Summary results from a generalized linear model of four climactic variables as predictors for the presence of diploid and tetraploid populations of *Rhodohypoxis baurii* var. *platypetala*. 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.

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 meters (°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)	-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 meters (°C) * Precipitation corrected (mm/day)	-0.03	0.001	-34.11	**0.0001**	0.9	0.97	0.97
Precipitation corrected (mm/day) * 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)	0.01	0.0002	43.31	**0.0001**	1.01	1.01	**1.01**

Table 3.5 Soil nutrient (nitrogen, phosphorus, and potassium) and pH results of samples that were collected from four natural populations (three diploid [2*x*], one tetraploid [4*x*]) of *Rhodohypoxis baurii* var. *platypetala* that occur across the KwaZulu-Natal (KZN) part of the Drakensberg grasslands, South Africa. 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 on Table 3.1 and Fig. 3.2.

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

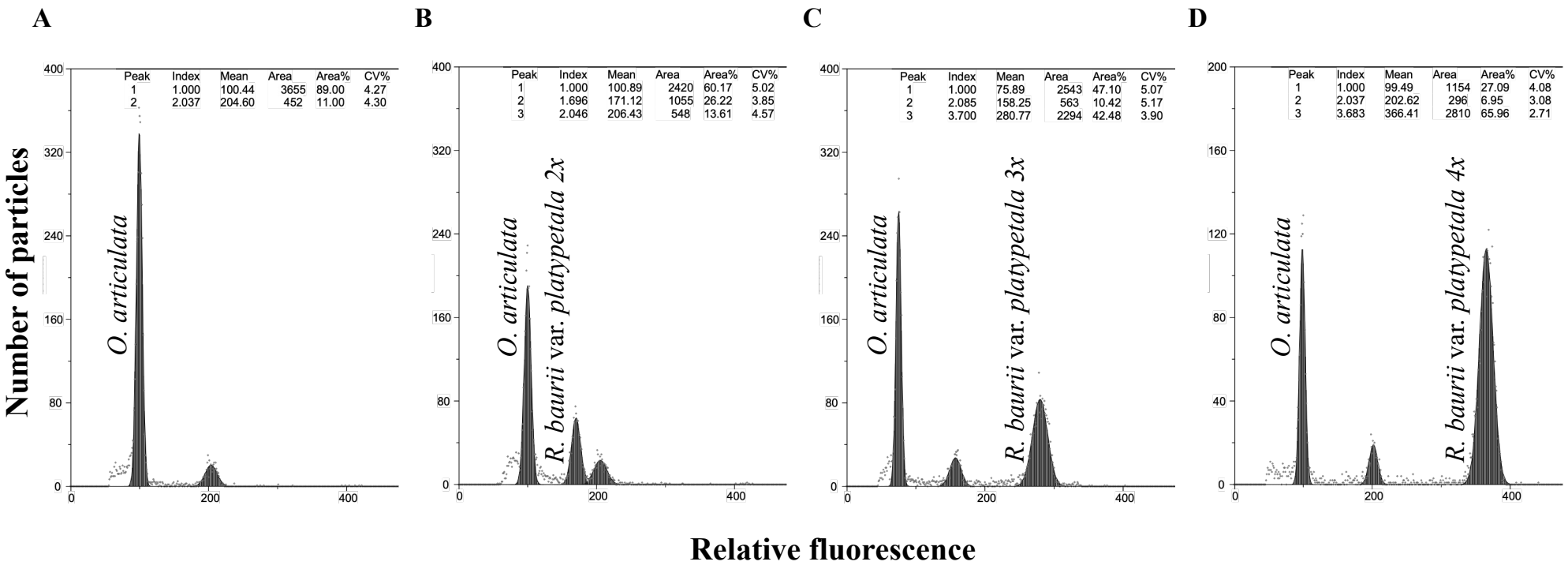
Figure Legend

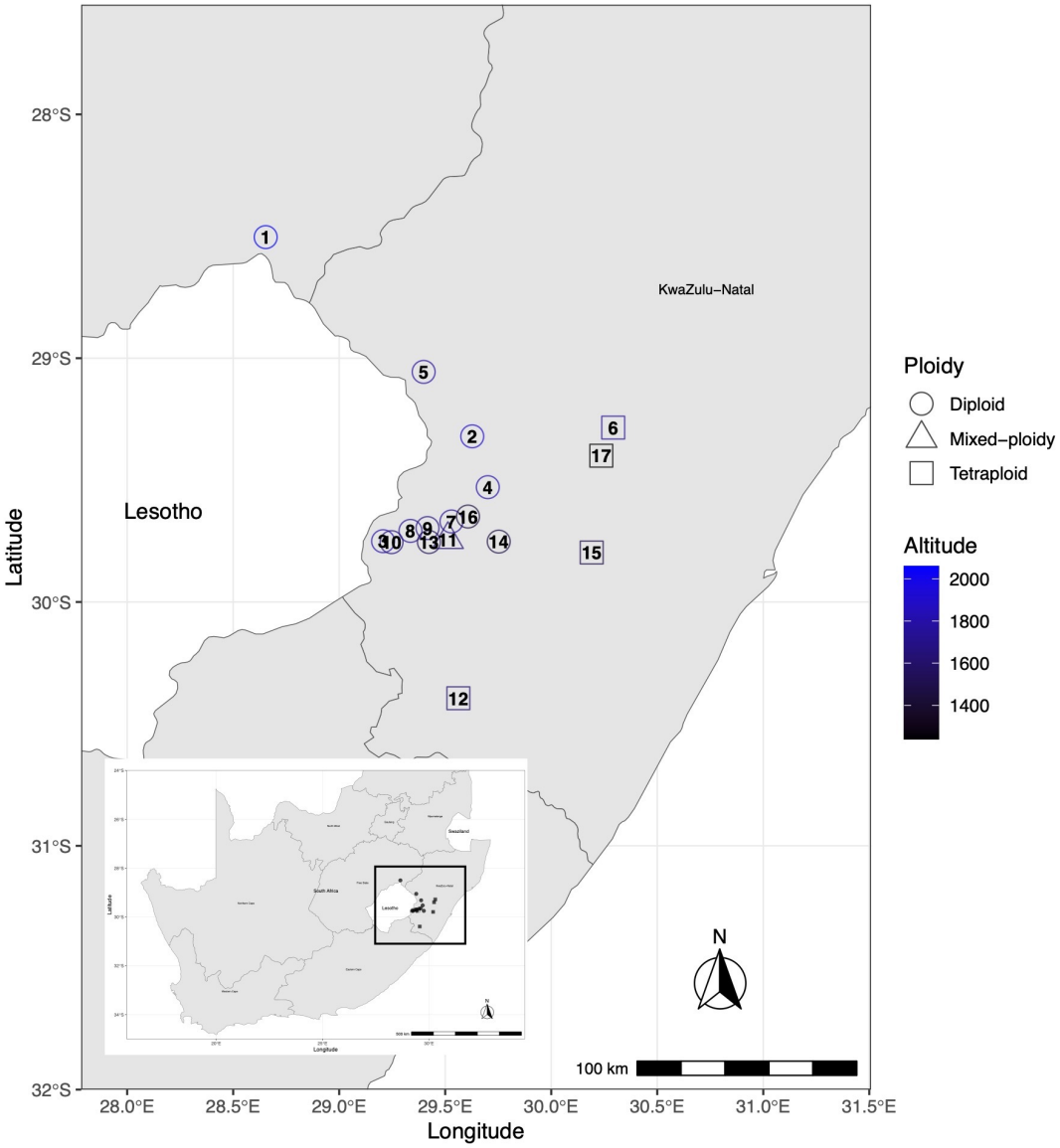
Fig. 3.1 Relative fluorescence histograms obtained from flow cytometry analyses of *Rhodohypoxis baurii* var. *platypetala* leaf samples that were used for this study. Ploidy level of the standard, *Oxalis articulata* (A), and three ploidy levels of *R. baurii* var. *platypetala* – diploid (B), triploid (C) and tetraploid (D), are shown.

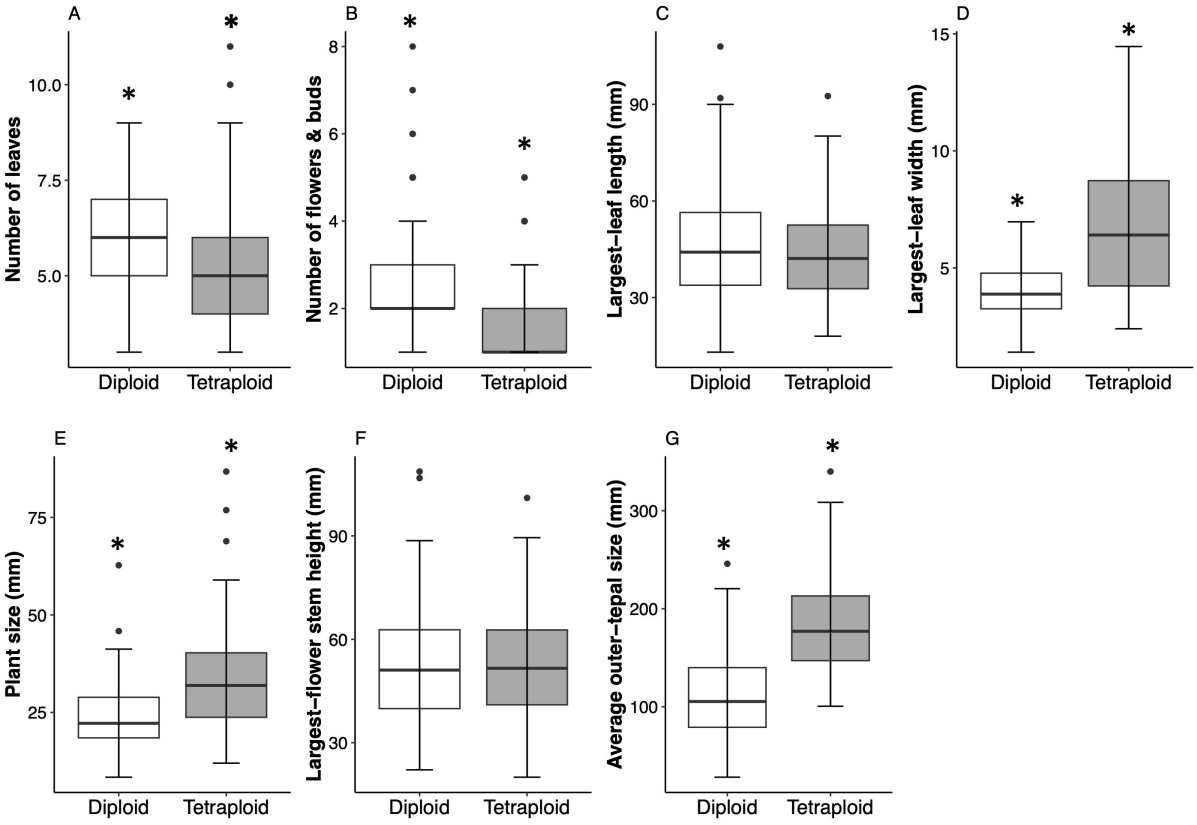
Fig. 3.2 A map of 17 sampled populations of *Rhodohypoxis baurii* var. *platypetala* in South Africa, and the distribution of three ploidy levels of this taxon that were identified across the Free State and KwaZulu-Natal parts of the Drakensberg grasslands, South Africa. Circles and squares represent diploid (2*x*) and tetraploid (4*x*) populations, respectively, and the mixed-ploidy population (2*x,* 3*x*) is represented by a triangle. The colour gradient represents the altitude (m a.s.l.) where each populations occurs. Population IDs correspond to the IDs on Tables 3.1 and 3.5.

Fig. 3.3 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.

Fig. 3.4 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.


A
B
C
D
Number of particles
Relative fluorescence
O. articulata
R. baurii var. platypetala 2x
R. baurii var. platypetala 3x
R. baurii var. platypetala 4x
Peak Index Mean Area Area% CV%
1 1.000 100.44 3655 89.00 4.27
2 2.037 204.60 452 11.00 4.30
Peak Index Mean Area Area% CV%
1 1.000 100.89 2420 60.17 5.02
2 1.696 171.12 1055 26.22 3.85
3 2.046 206.43 548 13.61 4.57
Peak Index Mean Area Area% CV%
1 1.000 75.89 2543 47.10 5.07
2 2.085 158.25 563 10.42 5.17
3 3.700 280.77 2294 42.48 3.90
Peak Index Mean Area Area% CV%
1 1.000 99.49 1154 27.09 4.08
2 2.037 202.62 296 6.95 3.08
3 3.683 366.41 2810 65.96 2.71


28°S
29°S
30°S
31°S
32°S
Latitude
28.0°E
28.5°E
29.0°E
29.5°E
30.0°E
30.5°E
31.0°E
31.5°E
Longitude
Lesotho
KwaZulu−Natal
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
N
100 km
Ploidy
Diploid
Mixed−ploidy
Tetraploid
Altitude
2000
1800
1600
1400


A
Number of leaves
10.0
7.5
5.0
Diploid
Tetraploid
B
Number of flowers & buds
8
6
4
2
Diploid
Tetraploid
C
Largest−leaf length (mm)
90
60
30
Diploid
Tetraploid
D
Largest−leaf width (mm)
15
10
5
Diploid
Tetraploid
E
Plant size (mm)
75
50
25
Diploid
Tetraploid
F
Largest−flower stem height (mm)
90
60
30
Diploid
Tetraploid
G
Average outer−tepal size (mm)
300
200
100
Diploid
Tetraploid


A
B
C
3
4

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Appendix S1 Summary of flow cytometric results of *Rhodohypoxis baurii* var. *platypetala* individuals that were sampled across the Drakensberg grasslands, South Africa. Mean fluorescence intensity (MFI) and coefficient of variation of the mean [CV (%)] are shown for the standard and corresponding sample. FS refers to Free State province, KZN refers to Kwa-Zulu Natal province.

Population	Sample ID	Standard G0/G1 peak MFI	Standard CV (%)	Sample G0/G1 peak MFI	Sample CV (%)
Minerva Nature Reserve, KZN	Min1	102.00	6.86	411.09	3.76
Minerva Nature Reserve, KZN	Min2	109.75	5.49	428.85	3.31
Minerva Nature Reserve, KZN	Min3	121.10	6.37	464.43	4.13
Minerva Nature Reserve, KZN	Min4	101.85	6.62	404.39	3.63
Minerva Nature Reserve, KZN	Min5	100.23	7.19	409.93	4.14
Minerva Nature Reserve, KZN	Min6	102.44	6.67	419.16	3.24
Minerva Nature Reserve, KZN	Min7	110.07	5.44	433.12	4.84
Minerva Nature Reserve, KZN	Min8	110.80	5.05	435.78	3.31
Minerva Nature Reserve, KZN	Min9	100.32	4.90	397.04	3.62
Minerva Nature Reserve, KZN	Min10	103.69	4.87	408.99	3.56
Minerva Nature Reserve, KZN	Min11	105.43	5.05	417.92	3.48
Minerva Nature Reserve, KZN	Min12	105.68	6.50	419.13	3.98
Minerva Nature Reserve, KZN	Min13	98.54	7.30	398.69	4.66
Minerva Nature Reserve, KZN	Min14	106.53	5.19	419.43	4.12
Minerva Nature Reserve, KZN	Min15	107.33	4.57	426.11	3.35
Minerva Nature Reserve, KZN	Min18	105.63	6.15	411.41	4.07
Mt Gilboa Nature Reserve, KZN	Mt1	112.89	5.20	440.40	3.25
Mt Gilboa Nature Reserve, KZN	Mt3	108.43	7.33	411.70	5.49
Mt Gilboa Nature Reserve, KZN	Mt5	114.68	7.12	438.76	5.24
Mt Gilboa Nature Reserve, KZN	Mt6	111.58	7.95	422.49	5.92
Mt Gilboa Nature Reserve, KZN	Mt7	107.26	8.00	412.20	5.73
Mt Gilboa Nature Reserve, KZN	Mt8	110.35	9.86	418.94	6.30

Mt Gilboa Nature Reserve, KZN	Mt9	108.38	7.12	414.53	5.34
Mt Gilboa Nature Reserve, KZN	Mt10	108.23	8.38	414.09	5.66
Mt Gilboa Nature Reserve, KZN	Mt11	110.33	7.02	422.65	5.02
Mt Gilboa Nature Reserve, KZN	Mt12	105.70	5.31	413.81	3.03
Mt Gilboa Nature Reserve, KZN	Mt13	111.61	6.46	418.71	5.23
Mt Gilboa Nature Reserve, KZN	Mt14	110.75	6.62	417.17	5.53
Mt Gilboa Nature Reserve, KZN	Mt15	115.42	7.35	435.17	5.77
Mt Gilboa Nature Reserve, KZN	Mt16	102.93	4.78	403.30	3.69
Mt Gilboa Nature Reserve, KZN	Mt17	102.39	4.26	399.12	2.95
Mt Gilboa Nature Reserve, KZN	Mt18	98.90	6.05	388.22	4.24
Mt Gilboa Nature Reserve, KZN	Mt19	109.00	5.06	425.28	3.11
Mt Gilboa Nature Reserve, KZN	Mt20	103.07	6.94	399.91	3.95
Mt Gilboa Nature Reserve, KZN	Mt21	111.42	5.66	431.03	3.60
Mt Gilboa Nature Reserve, KZN	Mt22	108.57	5.69	424.68	3.19
Mt Gilboa Nature Reserve, KZN	Mt23	107.43	5.09	421.66	3.12
Mt Gilboa Nature Reserve, KZN	Mt24	109.74	4.97	428.87	3.25
Mt Gilboa Nature Reserve, KZN	Mt25	104.79	6.09	408.02	3.75
Mt Gilboa Nature Reserve, KZN	Mt26	104.83	7.71	373.87	3.86
Howick, KZN	Hw1	103.03	5.09	367.07	4.20
Howick, KZN	Hw2	109.08	4.87	385.39	3.79
Howick, KZN	Hw3	99.89	5.03	386.79	3.22
Howick, KZN	Hw4	110.53	5.27	420.87	3.66
Howick, KZN	Hw5	103.14	5.63	396.11	3.28
Howick, KZN	Hw6	99.55	9.43	388.23	4.27
Howick, KZN	Hw7	101.43	5.54	391.14	3.41
Howick, KZN	Hw8	107.58	7.99	405.66	4.45
Howick, KZN	Hw9	107.56	5.41	413.16	3.61
Howick, KZN	Hw11	92.29	6.22	353.94	3.62
Howick, KZN	Hw14	102.66	7.34	409.21	4.03
Howick, KZN	Hw15	99.87	7.08	374.25	3.67
Howick, KZN	Hw16	101.48	5.35	397.14	3.34
Howick, KZN	Hw17	101.85	5.44	398.46	3.02
Howick, KZN	Hw18	108.64	5.51	416.86	3.57

Howick, KZN	Hw19	105.57	5.45	407.89	3.87
Howick, KZN	Hw20	110.19	4.97	424.24	3.83
Howick, KZN	Hw21	110.67	5.40	421.11	4.29
Howick, KZN	Hw22	109.34	4.40	425.28	3.02
Howick, KZN	Hw23	106.64	5.70	414.57	3.84
Howick, KZN	Hw24	108.43	4.40	424.69	3.32
Howick, KZN	Hw25	104.98	4.85	414.71	2.97
Howick, KZN	Hw26	103.12	5.52	400.29	3.05
Hebron, KZN	Heb1	103.12	6.83	411.91	4.13
Hebron Farm road, KZN	Heb16	98.69	5.15	392.11	2.73
Hebron Farm road, KZN	Heb17	75.89	5.07	280.77	3.90
Hebron Farm road, KZN	Heb18	80.84	8.71	305.03	4.51
Hebron Farm road, KZN	Heb19	95.80	5.52	353.09	4.72
Hebron Farm road, KZN	Heb20	99.49	4.08	366.41	2.71
Hebron Farm road, KZN	Heb21	106.27	4.15	411.91	4.13
Stoffelton, Ngqiya, KZN	St1	103.26	4.76	181.92	3.68
Stoffelton road, Ngqiya, KZN	St2	98.22	4.60	169.15	3.71
Stoffelton road, Ngqiya, KZN	St3	91.61	6.98	199.49	4.09
Stoffelton road, Ngqiya, KZN	St4	97.92	6.09	199.49	4.09
Stoffelton road, Ngqiya, KZN	St5	95.70	6.80	199.49	4.09
Stoffelton road, Ngqiya, KZN	St6	102.36	6.38	199.49	4.09
Stoffelton road, Ngqiya, KZN	St7	103.41	4.70	190.26	6.05
Stoffelton road, Ngqiya, KZN	St8	97.79	4.62	175.28	3.86
Stoffelton road, Ngqiya, KZN	St9	97.37	4.53	173.72	3.98
Stoffelton road, Ngqiya, KZN	St10	100.61	5.16	178.71	3.46
Stoffelton road, Ngqiya, KZN	St11	100.71	6.11	177.13	4.44
Stoffelton road, Ngqiya, KZN	St12	96.00	3.99	167.84	3.42
Stoffelton road, Ngqiya, KZN	St13	96.57	5.26	167.71	3.95
Stoffelton road, Ngqiya, KZN	St14	94.53	5.38	167.86	3.86
Stoffelton road, Ngqiya, KZN	St15	96.46	4.63	169.35	3.83
Stoffelton road, Ngqiya, KZN	St16	100.42	5.21	175.67	4.80
Stoffelton road, Ngqiya, KZN	St17	90.84	5.92	162.36	4.74
Stoffelton road, Ngqiya, KZN	St18	94.82	4.54	165.68	3.30

Stoffelton road, Ngqiya, KZN	St19	90.07	5.93	157.73	4.50
Underberg, KZN	Ub2	102.09	6.68	206.43	4.57
Underberg, KZN	Ub3	100.89	5.02	206.43	4.57
Underberg, KZN	Ub4	99.81	5.31	202.72	5.61
Underberg, KZN	Ub5	97.38	5.35	201.71	4.29
Underberg, KZN	Ub7	103.01	5.08	202.72	5.61
Underberg, KZN	Ub8	105.89	5.63	202.72	5.61
Underberg, KZN	Ub9	107.47	5.84	202.72	5.61
Underberg, KZN	Ubi1	100.41	6.60	202.72	5.61
Underberg, KZN	Ubi2	93.46	6.80	162.58	4.97
Underberg, KZN	Ubi3	98.43	9.84	171.94	4.35
Underberg, KZN	Ubi4	102.77	5.53	171.94	4.35
Underberg, KZN	Ubi5	98.44	4.37	171.94	4.35
Underberg, KZN	Ubi7	95.39	3.36	195.45	3.87
Underberg, KZN	Ubi8	94.88	5.47	195.45	3.87
Drakensberg Gardens, KZN	Dg1	70.27	8.53	128.29	5.68
Drakensberg Gardens, KZN	Dg2	86.03	6.30	162.70	7.23
Drakensberg Gardens, KZN	Dg3	77.76	5.97	141.90	4.62
Drakensberg Gardens, KZN	Dg5	105.55	6.27	193.65	3.96
Drakensberg Gardens, KZN	Dg6	96.88	7.39	212.56	6.69
Drakensberg Gardens, KZN	Dg7	99.14	7.70	198.37	7.56
Sunset Farm, KZN	Sun1	97.86	8.50	220.20	4.78
Sunset Farm, KZN	Sun2	89.56	9.35	220.20	4.78
Sunset Farm, KZN	Sun3	89.26	7.10	220.20	4.78
Sunset Farm, KZN	Sun4	102.50	7.66	220.20	4.78
Sunset Farm, KZN	Sun5	106.85	9.83	211.74	9.91
Sunset Farm, KZN	Sun16	108.22	9.69	335.34	7.71
Sunset Farm, KZN	Sun18	105.07	4.86	317.79	3.31
Sunset Farm, KZN	Sun19	105.44	5.07	317.79	3.31
Sunset Farm, KZN	Sun20	114.46	4.30	220.20	4.78
Sunset Farm, KZN	Sun21	94.86	8.09	189.17	7.17
Sunset Farm, KZN	Sun22	88.91	8.36	178.26	9.09
Sunset Farm, KZN	Sun23	82.32	9.55	166.45	9.97

Sunset Farm, KZN	Sun24	108.81	7.02	220.20	4.78
Sunset Farm, KZN	Sun25	103.35	8.06	220.20	4.78
Sunset Farm, KZN	Sun26	98.41	7.95	220.20	4.78
Sunset Farm, KZN	Sun27	96.59	6.78	190.44	5.37
Sunset Farm, KZN	Sun28	96.74	7.12	220.20	4.78
Sunset Farm, KZN	Sun29	104.23	5.86	220.20	4.78
Sunset Farm, KZN	Sun30	102.90	5.51	203.40	5.27
Sunset Farm, KZN	Sun31	102.81	7.45	173.51	4.27
Sunset Farm, KZN	Sun32	110.45	8.07	183.09	5.06
Sunset Farm, KZN	Sun33	104.71	7.04	265.24	4.26
Sunset Farm, KZN	Sun34	106.59	8.11	188.42	7.12
Highmoor Nature Reserve, KZN	Hig1	97.93	4.68	183.54	4.55
Highmoor Nature Reserve, KZN	Hig2	83.19	4.68	154.10	3.84
Highmoor Nature Reserve, KZN	Hig10	96.41	7.91	194.72	7.70
Golden Gate Highlands National Park, FS	Gg1	100.28	5.25	191.14	3.93
Golden Gate Highlands National Park, FS	Gg2	94.43	8.19	180.50	6.97
Golden Gate Highlands National Park, FS	Gg4	99.58	5.93	182.80	4.32
Golden Gate Highlands National Park, FS	Gg6	104.02	6.42	192.97	4.81
Golden Gate Highlands National Park, FS	Gg7	99.08	7.45	191.67	8.90
Golden Gate Highlands National Park, FS	Gg8	100.05	7.38	192.62	7.68
Golden Gate Highlands National Park, FS	Gg10	104.59	7.64	189.41	4.94
Golden Gate Highlands National Park, FS	Gg11	102.24	7.88	196.26	8.38
Golden Gate Highlands National Park, FS	Gg26	110.19	7.43	204.15	6.85
Cobham Nature Reserve, KZN	Cob1	97.43	7.38	182.78	5.45
Cobham Nature Reserve, KZN	Cob3	104.70	6.13	204.63	5.27
Cobham Nature Reserve, KZN	Cob4	103.19	6.18	191.32	4.30
Cobham Nature Reserve, KZN	Cob5	100.08	7.51	184.57	4.44
Cobham Nature Reserve, KZN	Cob6	92.87	6.35	231.68	4.66
Cobham Nature Reserve, KZN	Cob7	96.19	8.49	178.45	5.74
Cobham Nature Reserve, KZN	Cob8	95.41	6.55	182.17	5.00
Cobham Nature Reserve, KZN	Cob9	103.03	5.87	191.86	4.57
Cobham Nature Reserve, KZN	Cob10	103.46	6.12	199.70	5.19
Cobham Nature Reserve, KZN	Cob14	94.29	9.47	186.48	6.28

Bushman's Nek, KZN	Bsh6	98.30	7.97	168.94	5.10
Bushman's Nek, KZN	Bsh8	99.31	6.31	206.43	4.57
Bushman's Nek, KZN	Bsh11	92.63	7.33	206.43	4.57
Bushman's Nek, KZN	Bsh13	104.92	8.69	184.21	5.40
Bushman's Nek, KZN	Bsh16	96.98	7.07	168.52	6.40
Bushman's Nek, KZN	Bsh18	82.56	8.85	184.21	5.40
Giants Castle Game Reserve, KZN	Gc20	113.79	8.02	215.12	8.57
Underberg, KZN	Ubii1	98.56	5.31	186.09	9.78
Underberg, KZN	Ubii2	101.79	4.58	181.43	3.60
Underberg, KZN	Ubii3	102.13	5.19	209.42	3.48
Underberg, KZN	Ubii4	94.35	9.92	209.42	3.48
Underberg, KZN	Ubii5	91.03	4.72	160.38	3.87
Underberg, KZN	Ubii6	99.58	6.03	187.41	4.21
Underberg, KZN	Ubii7	96.07	4.55	164.86	3.97
Underberg, KZN	Ubii8	87.85	5.90	181.99	4.62
Underberg, KZN	Ubii10	90.92	7.43	181.99	4.62
Underberg, KZN	Ubii11	91.86	5.65	181.99	4.62
Underberg, KZN	Ubii12	99.09	5.07	202.77	3.72
Underberg, KZN	Ubii13	91.55	5.34	159.59	4.56
Underberg, KZN	Ubii14	92.48	5.93	190.17	3.96
Underberg, KZN	Ubii16	97.82	5.30	172.33	3.73
Ingwe Local Municipality, KZN	Ing1	107.36	6.42	188.25	4.76
Ingwe Local Municipality, KZN	Ing2	101.72	6.41	181.72	4.56
Ingwe Local Municipality, KZN	Ing3	90.59	5.74	154.02	3.60
Ingwe Local Municipality, KZN	Ing4	99.41	8.81	173.64	7.99
Ingwe Local Municipality, KZN	Ing5	94.31	6.25	161.91	4.35
Ingwe Local Municipality, KZN	Ing8	91.57	7.85	154.38	5.41
Ingwe Local Municipality, KZN	Ing9	91.86	9.95	161.50	9.56
Ingwe Local Municipality, KZN	Ing10	103.52	6.15	178.81	5.56
Ingwe Local Municipality, KZN	Ing11	108.45	5.23	188.43	4.39
Ingwe Local Municipality, KZN	Ing13	102.65	5.63	176.52	4.30
Ingwe Local Municipality, KZN	Ing14	91.74	6.78	158.57	6.15
Ingwe Local Municipality, KZN	Ing17	74.69	9.19	129.78	8.66

Ingwe Local Municipality, KZN	Ing18	93.93	6.02	163.26	5.31
Lower Lotheni road, KZN	Low1	97.49	6.47	184.20	9.13
Lower Lotheni road, KZN	Low4	95.35	7.02	178.59	9.64
Lower Lotheni road, KZN	Low5	90.78	6.07	160.93	5.85
Lower Lotheni road, KZN	Low6	104.14	5.46	184.13	3.97
Lower Lotheni road, KZN	Low11	99.96	7.67	175.69	5.73
Lower Lotheni road, KZN	Low15	100.89	5.02	206.43	4.57
Lower Lotheni road, KZN	Low16	102.09	6.68	206.43	4.57

Appendix S2 Sample/standard fluorescence ratios are shown for each ploidy level of *Rhodohypoxis baurii* var. *platypetala* individuals that were sampled across the Drakensberg grasslands, South Africa. Letters denote groupings of Kruskal-Wallis multiple comparison post hoc tests where groups that do not significantly differ at $P = 0.05$ are marked with the same letter.

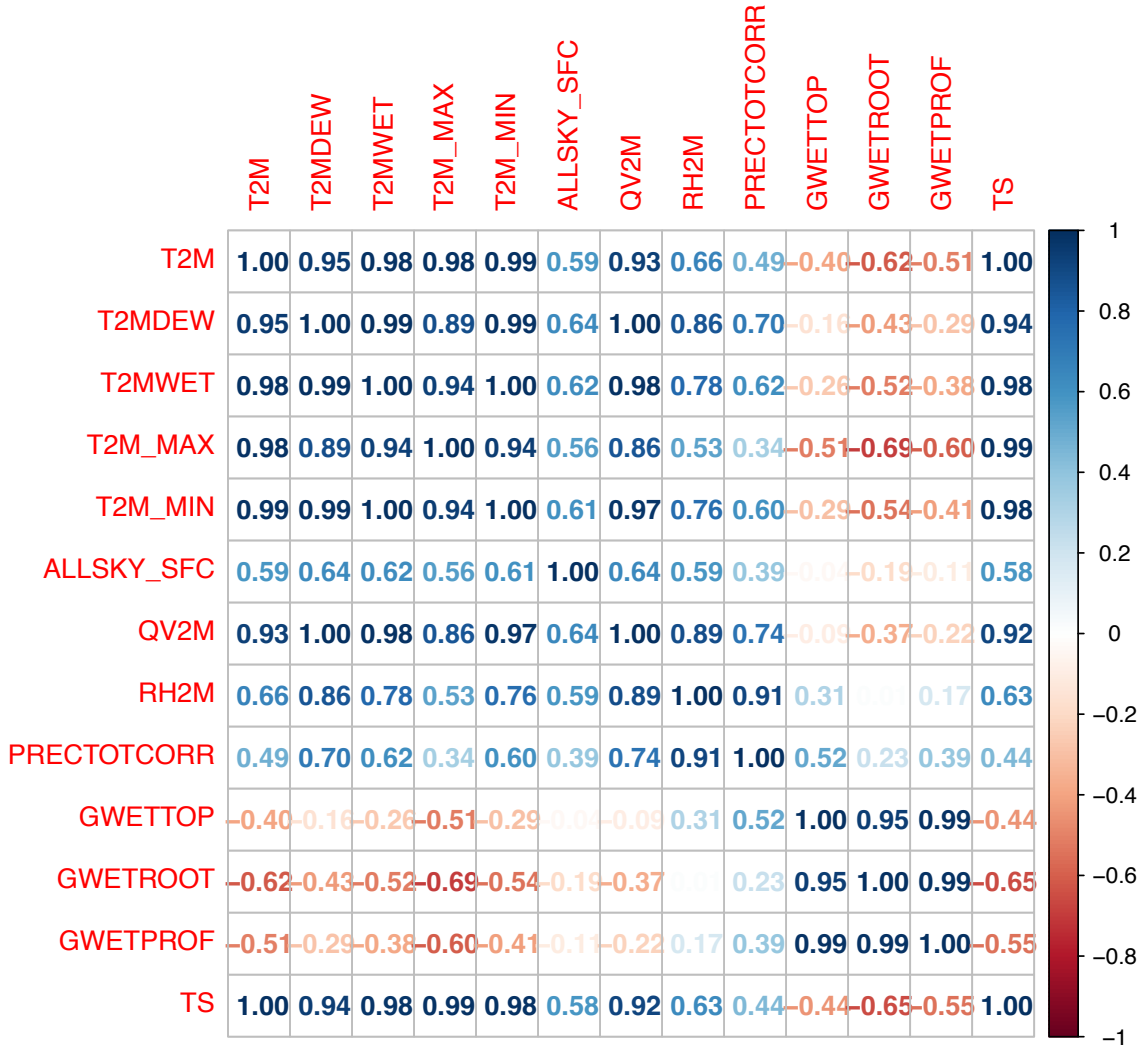
Ploidy	Relative fluorescence intensity (mean ± SE)	Number of populations/number of individuals
2*x*	1.91 ± 0.02 [a]	12/121
3*x*	2.92 ± 0.13 [ab]	1/4
4*x*	3.87 ± 0.01 [b]	4/70

Appendix S3 Correlation coefficients and significant results of the relationship between *Rhodohypoxis baurii* var. *platypetala* traits and climatic variables. T2M – temperature at 2 meters above the surface of the Earth (°C), ALLSKY_SFC – all sky surface longwave downward irradiance (horizontal infrared radiation intensity from sky; W m^{-2}), PRECTOTCORR – precipitation corrected (mm/day), and GWETPROF – profile soil moisture (0–1). *P*-values are presented in parentheses, where significant results are bolded.

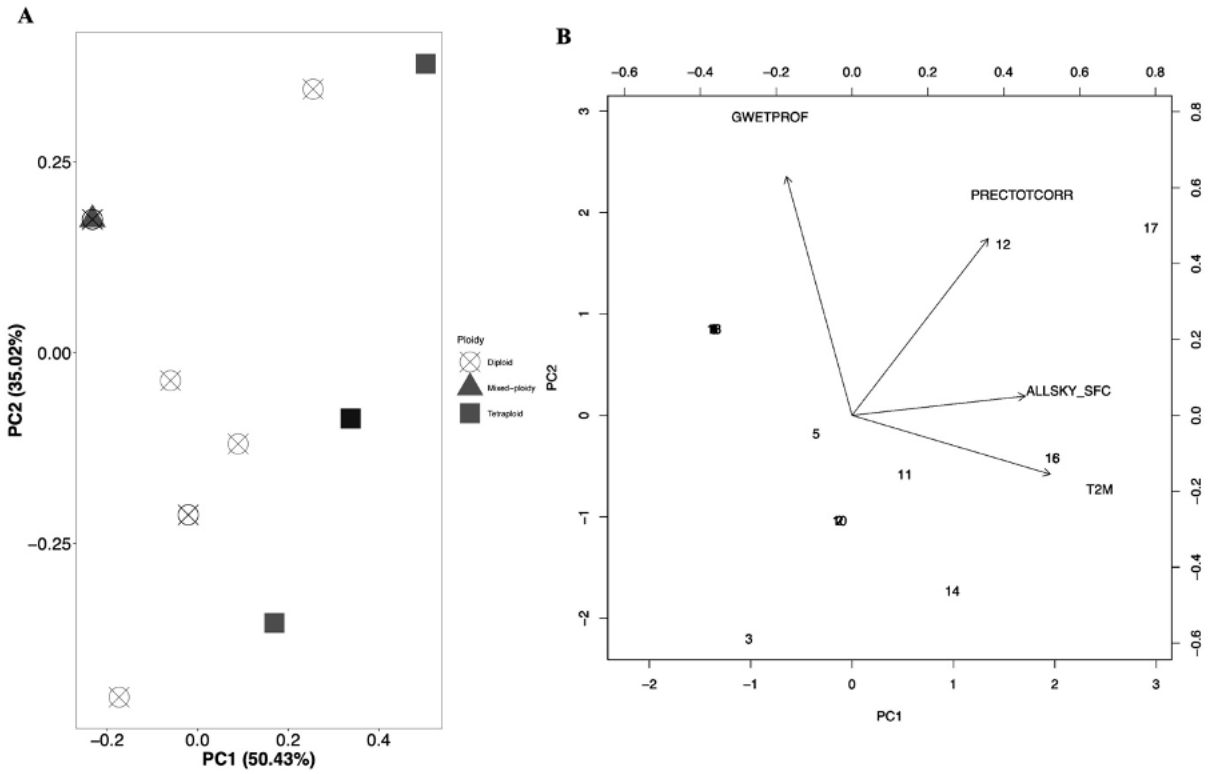
Variable	Largest-leaf width (mm)	Number of flowers and buds	Largest-flower tepal size (mm)	Largest-flower stem height (mm)
T2M	0.38 (0.63)	-0.50 (0.51)	0.73 (0.11)	0.17 (0.66)
ALLSKY_SFC	0.38 (0.62)	-0.65 (0.18)	0.87 (**0.01**)	-0.10 (0.80)
PRECTOTCORR	0.36 (0.68)	-0.64 (0.19)	0.85 (**0.02**)	-0.07 (0.86)
GWETPROF	-0.48 (0.75)	-0.05 (1)	-0.06 (1)	-0.17 (1)

Appendix S4 Mean ± SE of 13 climactic variables compared among diploid (2*x*), mixed-ploidy (*2x*, 3*x*) and tetraploid (4*x*) populations of *Rhodohypoxis baurii* var. *platypetala*. Letters denote groupings of Kruskal-Wallis multiple comparison post hoc tests where groups that do not significantly differ at $P = 0.05$ are marked with the same letter.

	Mean ± SE		
Variable	2*x*	Mixed-ploidy	4*x*
Temperature at 2 meters (°C)	12.92 ± 0.50 [a]	11.18 ± 0.08 [b]	15.39 ± 0.19 [c]
Dew/Frost point at 2 meters (°C)	4.49 ± 0.65 [a]	2.73 ± 0.11 [b]	8.10 ± 0.57 [c]
Wet bulb temperature at 2 meters (°C)	8.71 ± 0.57 [a]	6.96 ± 0.09 [b]	11.74 ± 0.37 [c]
Earth skin temperature (°C)	13.10 ± 0.48 [a]	11.42 ± 0.09 [b]	15.49 ± 0.11 [c]
Temperature at 2 meters maximum (°C)	20.98 ± 0.50 [a]	19.18 ± 0.08 [b]	23.32 ± 0.23 [c]
Temperature at 2 meters minimum (°C)	6.59 ± 0.56 [a]	4.78 ± 0.08 [b]	9.46 ± 0.38 [c]
Longwave downward irradiance (W m^{-2})	310.40 ± 0.09 [a]	310.50 ± 0.70 [a]	339.01 ± 6.05 [b]
Specific humidity at 2 meters (g kg^{-1})	7.14 ± 0.23 [a]	6.57 ± 0.05 [b]	8.39 ± 0.24 [c]
Relative humidity at 2 meters (%)	63.31 ± 0.93 [a]	62.90 ± 0.25 [a]	67.82 ± 1.48 [b]
Precipitation corrected (mm/day)	2.83 ± 0.11 [a]	2.82 ± 0.09 [a]	3.14 ± 0.26 [b]
Surface soil wetness (0–1)	0.60 ± 0.01 [a]	0.64 ± 0.002 [b]	0.58 ± 0.02 [c]
Root zone soil wetness (0–1)	0.62 ± 0.01 [a]	0.66 ± 0.001 [b]	0.58 ± 0.02 [c]
Profile soil moisture (0–1)	0.62 ± 0.01 [a]	0.65 ± 0.001 [b]	0.59 ± 0.02 [c]



Appendix S5 Correlation matrix of 13 climatic variables assigned for the 17 sampled populations of *Rhodohypoxis baurii* var. *platypetala.* Four uncorrelated climatic variables selected using a correlation coefficient cutoff of < 0.60 for further analyses: T2M – temperature at 2 meters above the surface of the Earth (°C), ALLSKY_SFC – all sky surface longwave downward irradiance (horizontal infrared radiation intensity from sky; W m $^{-2}$), PRECTOTCORR – precipitation corrected (mm/day), and GWETPROF – profile soil moisture (0–1).



Appendix S6 PCA of *Rhodohypoxis baurii var. platypetala* populations according to ploidy level and climatic variables (A). Four climactic variables were chosen for the PCA to assess their associations with ploidy level distributions: T2M – temperature at 2 meters above the surface of the Earth (°C), ALLSKY_SFC – all sky surface longwave downward irradiance (horizontal infrared radiation intensity from sky; W m $^{-2}$), PRECTOTCORR – precipitation corrected (mm/day), and GWETPROF – profile soil moisture (0–1; B).

Appendix S7 Summary of PCA results of four climatic variables that were tested for associations with ploidy level distributions: T2M – temperature at 2 meters above the surface of the Earth (°C), ALLSKY_SFC – all sky surface longwave downward irradiance (horizontal infrared radiation intensity from sky; W m^{-2}), PRECTOTCORR – precipitation corrected (mm/day), and GWETPROF – profile soil moisture (0–1).

	PC1	PC2
Factor loadings		
T2M	0.65	-0.19
ALLSKY_SFC	0.57	0.06
PRECTOTCORR	0.45	0.58
GWETPROF	-0.22	0.79
Eigenvalue	2.02	1.40
Proportion of variance explained	0.50	0.35

CHAPTER 4

DO TETRAPLOIDS DEMONSTRATE GREATER SURVIVORSHIP OR LARGER MORPHOLOGICAL TRAITS AND INCREASED REPRODUCTIVE OUTPUT UNDER DIFFERENT SOIL CONDITIONS?

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Prepared for submission to *Journal of Experimental Botany*

Abstract

The response of plants to environmental factors is key in facilitating plant growth in natural environments. For instance, nitrogen (N) and phosphorus (P) are important for cell growth and nucleic acids. The available quantity of these nutrients may restrict where plants, particularly polyploid individuals, can establish, grow, and persist. Polyploidy is known to alter plant phenotypes and may enhance the ability of plants to respond to a range of soil environments. Here, we used growth chambers and reciprocal transplants to test whether different soil conditions (N, P, and salinity) and other environmental factors in both field and greenhouse environments affected morphological traits, reproductive output, and survival of diploid and tetraploid individuals of the red stars (*Rhodohypoxis baurii* var. *platypetal*a, Hypoxidaceae). Previous work on *R. baurii* var. *platypetal*a found that tetraploids generally occurred in soil with marginally higher N and P than diploids in the field, and that environmental variables, such as precipitation, correlated with changes in morphological traits. In addition to the experimental treatments, we tested whether growing site (greenhouse or field) significantly affected the traits. We found that tetraploids had wider leaves whereas diploid individuals had a greater number of leaves and flowers regardless of growing site. In the growth chamber treatments (N, P, and salinity), tetraploidy was not associated with larger morphological traits and increased reproductive output under N or P enrichment, nor increased survival rate under saline conditions. Reciprocal transplant findings showed a similar survivorship and reproductive output between diploid and tetraploid individuals, suggesting that the ploidy levels are capable of establishing in each other's environment. Together, findings from this study indicate that increased ploidy in this taxon does not necessarily confer an advantage or limitation under different treatments (N, P, and salinity) and field environmental conditions.

Key words: Drakensberg grasslands, Hypoxidaceae, polyploidy, phosphorus, reciprocal transplants, *Rhodohypoxis baurii*

INTRODUCTION

Plants respond to changing environmental conditions, like variable moisture availability or nutrient limitation, in order to grow, establish, and persist (Picotte *et al*., 2007; Guan *et al*., 2011; Mtileni *et al*., 2024). Polyploidy, a widespread condition of possessing more than two sets of chromosomes, may enhance the ability of plants to respond to different environmental conditions (Maherali *et al*., 2009; Zhang *et al*., 2010; Liu *et al*., 2011; Chao *et al*., 2013; Yang *et al*., 2014) due to mechanisms such as increased heterozygosity, phenotypic changes due to increased cell size, and increased adaptive potential (for review, see te Beest *et al*., 2012). As a result, polyploid plants may show trait divergence across different environments (Wei *et al*., 2019), broad ecological tolerance (Husband *et al*., 2013) and adaptation to novel environments (Ramsey, 2011). Polyploidy may also provide additional challenges due to higher nutrient requirements after whole genome duplication (Leitch and Leitch, 2008). For instance, polyploid plants may perform better than diploids, through root growth and reproduction, when nitrogen and phosphorus levels are higher (Guignard *et al*., 2016; Walczyk and Hersch-Green, 2019; Anneberg and Segraves, 2020).

Consequences of polyploidization per se are often difficult to disentangle, as polyploid outcomes may be compensated by the general adaptive ability of plants to survive and grow in their environments. For instance, polyploidization does not always lead to niche divergence between diploids and polyploids in natural environments (Glennon *et al*., 2014; Marchant *et al*., 2016; but see Thompson *et al*., 2014; Visger *et al*., 2016) and there may not always be a relationship between polyploid plants and their response to environmental change or stress (Wei *et al*., 2020; Mtileni *et al*., 2021; Harms *et al*., 2021). For instance, a similar water use efficiency and gas exchange sensitivity has been reported for diploids and tetraploids under water stress (Maherali *et al*., 2009; Mtileni *et al*., 2021). Therefore, comparing growth and reproduction of polyploid and diploid individuals can be informative

for understanding whether the interaction of environmental variables and genetic context (e.g., genotypes) may predict the successful establishment of polyploids, although it is often unclear whether differences in traits and responses to environmental change between ploidy levels is due to polyploid outcomes alone or adaptive evolution after the polyploidy event. Recently, Wei *et al*. (2020) found that genome doubling alone affected traits in *Fragaria*, but not stress tolerance.

Polyploids are thought to show high resistance to several environmental factors, like drought, heat, cold, and salt (Bomblies, 2020). For instance, polyploids in Cape schoenoid sedges have been found to exhibit potentially higher water use efficiency than diploids under water stress due to possessing larger but fewer stomata (van Mazijk *et al*., 2024; but see Mtileni *et al*., 2021). When plants are exposed to saline conditions, the inner-outer osmotic gradient of the cell may increase as the plants accumulate salt, leading to dehydrated cells and altering plant growth (Volkmar *et al*., 1998). There are polyploid plants found to accumulate less salt through mechanisms such as elevated potassium uptake and stronger root sodium retention capacity compared to diploids (e.g., *Arabidopsis thaliana*, Chao *et al*., 2013; Yang *et al*., 2014). Polyploid plants have also been found to be more salt tolerance due to changes in the expression of genes involved in stress (e.g., *Triticum aestivum*, Yang *et al*., 2014).

How polyploidization affects a plant is likely to be species-specific. Experimental work at local scales (e.g., common garden experiments) has been useful in associating polyploidization with changes in plant traits in different environmental conditions, or lack thereof, with implications for plant establishment and distribution. Mtileni *et al*. (2021) used diploid and tetraploid individuals of *Rhodohypoxis baurii* to test if the ploidies responded differently under well-watered and water stress conditions, and they found that while ploidy affected leaf traits (e.g., larger stomata in tetraploids), it did not affect responses to water

stress. Other experiments that focused on saline conditions demonstrated that *Triticum aestivum* hexaploids exhibited beneficial traits such as increased germination rate compared to their progenitors under saline conditions and that polyploid plants are generally salt tolerant (Yang *et al*., 2014). Further, polyploids are hypothesized to have higher requirements for nitrogen and phosphorus than diploids after whole genome duplication (Leitch and Leitch, 2008; Faizullah *et al*., 2021) due to the larger number of nucleotides needed to build a larger genome. Working with *Botumus umbellatus*, Harms *et al*. (2021) investigated if triploids were favoured in nutrient enrichment over diploids and they found that higher ploidies did not support greater changes in traits or increase their reproductive output. In other studies, the trend of high requirements of nutrients for polyploid plants was supported as individuals with increased ploidy produced higher above-ground biomass under increased nitrogen and phosphorus in 60 species (Guignard *et al*., 2016). In *Arabidopsis thaliana*, nutrient availability affected ploidies differently as tetraploids grew larger and had a higher fitness than diploids under increased nitrogen and phosphorus (Anneberg and Segraves, 2020).

This study focuses on *Rhodohypoxis baurii* (Baker) Nel. var. *platypetala* (Asparagales: Hypoxidaceae), a Drakensberg near-endemic taxon of *R. baurii* (Hilliard and Burtt, 1978) which comprises diploids, triploids, and tetraploids across its native range in the Drakensberg grasslands (Saito, 1975; Mtileni *et al*., 2024) to establish whether increased ploidy affects the response of plants to different environmental conditions. In this taxon, the tetraploids generally have wider leaves and larger flowers than diploids (Mtileni *et al*., 2024) as well as larger stomata than diploids (Mtileni *et al.,* 2021). These traits coincide with research highlighting that polyploidy increases cell size and subsequently alters morphological and physiological traits (Masterson, 1994; Otto and Whitton, 2000; Ramsey and Ramsey, 2014; Doyle and Coate, 2019) which are often larger in polyploids (Yu *et al*., 2010; Liu *et al*., 2011; Wei *et al*., 2019, 2020; Porturas *et al*., 2019; Domínguez-Delgado *et*

al., 2021). Although previous work found morphological differences between ploidies in this taxon, both the locality of populations and ploidy affected leaf and flower traits such as largest-leaf width and largest-flower tepal size, and precipitation and horizontal infrared radiation intensity were significantly correlated with the differences in traits (Mtileni *et al*., 2024). Further research is necessary to test whether differences in traits between diploids and tetraploids are generally associated with changes in ploidy level or environmental conditions, like soil nutrients, at different growing sites.

Previous work also found that diploids and tetraploids of *R. baurii* var. *platypetala* did not generally co-occur in the field, and tetraploid individuals tended to occur in soil with slightly higher phosphorus and nitrogen than diploids (Mtileni *et al*., 2024). We then hypothesized that individuals of the two ploidy levels might show different traits (e.g., larger growth forms and higher composite fitness in polyploids) under greater nutrient availability due to higher demand for nitrogen and phosphorus in polyploids to build larger genomes (e.g., Guignard *et al*., 2016; Walczyk and Hersch-Green, 2019; Anneberg and Segraves, 2020). This taxon also occurs in the montane and alpine grasslands, where a soil survey indicated that there is some variation in electrical conductivity and exchangeable sodium within the grasslands (Nell and van Huyssteen, 2016). Therefore, we hypothesized that salinity might potentially be a general environmental variable that facilitates where different ploidies can occur. We conducted two experiments to test our hypotheses: a growth chamber experiment and reciprocal transplants. We hypothesized that different soil conditions (nitrogen, phosphorus, and salinity) in the greenhouse and field environmental conditions affected the taxon morphological traits, reproductive output (seeds and stolons), and survival of diploid and tetraploid individuals.

METHODS

Study system

Rhodohypoxis is a small genus of six species of herbaceous perennial geophytes that occur across the mountain grasslands of the KwaZulu-Natal Drakensberg in South Africa, the Lesotho plateau, and into Eswatini (Hilliard and Burtt, 1978). *Rhodohypoxis* plants die back in January–February and leaves and flowers emerge from the soil in mid–October, after the first rains of the summer rainfall. Polyploidy exists within this genus, with polyploid plants generally having larger stomata and higher trichome density than diploids (Mtileni *et al.,* 2021), but the response of polyploid plants to different environmental conditions remains unclear for this genus.

Plant collection in the field

Rhodohypoxis baurii var. *platypetala* individuals (n ≈ 110) were collected from seven populations across their natural distribution in the KwaZulu-Natal and Free State provinces, South Africa, over two years (2017 and 2018). During each collection, individuals were dug out and transplanted into separate 10 cm diameter plastic pots without washing off rhizosphere soil. Pots were subsequently supplemented with the Culterra potting mix containing 50% topsoil and 50% compost, after which all individuals were housed and maintained in the greenhouse at the University of the Witwatersrand, Johannesburg, South Africa. In the greenhouse, the individuals grew under 24-hour cycle ambient light conditions and experienced temperatures ranging between 10 and 30 °C. Each individual received ~200 mL of water per day through automated irrigation by sprinklers. Ploidy level for each individual was estimated using flow cytometry following Doležel *et al*. (2007) for another study (Ferreira *et al*., in prep). Genetic diversity was found to be similar among the sampled populations and genetic differentiation was low (D.E. Beukes, S. Archibald, K.L. Glennon; unpublished data), but genotype data were not available for all individuals included in this

study. In the growth chamber experiment (see below), we used these previously collected individuals from different populations that were housed in the same greenhouse to potentially remove confounding effects due to local adaptation to field habitats. We were unable to use seeds and seedlings or synthetic autotetraploids in the growth chambers as our pilot studies have not yielded mature seedlings after seed germination. This taxon also reproduces via underground stolons (Hilliard and Burtt, 1978) and it appears that seedling recruitment via seed germination is unlikely the prominent mode of population growth, but more data are needed to test this observation.

Field and greenhouse trait measurements

In November 2022, 108 individuals (53 diploids, 55 tetraploids) were selected from the collected stock and used in our growth chamber experiment (see below). The collected plants lived four to five years in the greenhouse before the experiment started, under the same environmental conditions as mentioned above. The following traits were measured on all individuals: total number of leaves and flowers (including buds and/or senesced flowers), and largest-leaf length (leaf blade tip to leaf base, mm) and corresponding central leaf width (mm), after which the individuals were moved to the growth chambers a week before the experiment started so they could acclimate to the chamber conditions. The trait measurements taken during this week were used as baseline measurements for our growth chamber experiment and to assess if the morphological differentiation between diploid and tetraploid individuals was present regardless of growing site. We compared the greenhouse measurements to field measurements that were taken in November 2021 on individuals from the same populations. Measurements were taken using digital calipers (GV9371, 150 mm, Grip) which were reset to zero after each measurement. Separate linear mixed-effects models were constructed using the *lme4* and *lmerTest* packages (Bates *et al*., 2015; Kuznetsova *et al*., 2017) to test for the effect of ploidy and growing site (field or greenhouse) on each trait.

Response variables were log-transformed to improve normality. Population was included as a random effect. A significant effect of the interaction of ploidy and growing site would indicate that plants of different ploidies differed in the traits under the two growing sites which represented different environmental conditions such as changes in water availability. All statistical analyses in this study were conducted in R version 4.2.1 (R Core Team, 2022).

Soil conditions and plant growth

The experiment was conducted in three reach-in growth chambers (CONVIRON; GEN1000) at the University of the Witwatersrand, Johannesburg, and ran for a total of six weeks between November and December 2022. The timeframe of the experiment corresponded to the growing season of the taxon in the field. Working in the growth chambers enabled us to assess the effect of our focal variables (nitrogen, phosphorus, and sodium chloride [NaCl]), while controlling for light, temperature, humidity, and pests. In each chamber, relative humidity and CO_2 were maintained at controlled set-points of 60% and 400 ppm, respectively, and temperature simulated daily changes in the greenhouse environment – i.e., 25 °C during the day (6h00–18h00) and 18 °C at night (18h00–6h00). The same incandescent and fluorescent lights were used in all three chambers for consistency, and they were all set to 100% intensity during the day to simulate a sunny conditions and turned off at night.

Three treatments (nitrogen, phosphorus, and NaCl) were used for the experiment where each treatment comprised different solutions of the focal nutrient and NaCl. The application of the three treatments was replicated across the three chambers to accommodate all plants used in the experiment. For the purpose of this study, we refer to the solutions within each of the three treatments (nitrogen, phosphorus, and NaCl) as ‘concentrations’ (e.g., high nitrogen, medium nitrogen , low nitrogen). Nitrogen was applied to plant soil as the substance urea (Duda Energy, United States) and phosphorus as superphosphate

(GROMOR (PTY) Ltd, South Africa). Each treatment substance (urea, superphosphate, and NaCl) was separately diluted in 1L of plain tap water, and each individual was watered by hand and received the same amount of nutrient or NaCl water every second day of the week, depending on the concentration of nitrogen, phosphorus, or NaCl assigned to the individual (e.g., 1L nutrient water / total number of individuals in low nitrogen concentration (12) = ~84 mL; see specific details below). All plants received equal amounts of potassium (250 mg L^{-1} ; applied as potassium sulphate) along with each nitrogen and phosphorus concentration. Our nutrient treatments, although designed to reflect a concentration gradient (low, medium, high), were chosen to coincide with the range of nitrogen, phosphorus, and potassium contents found in field populations of this taxon. In the nitrogen treatment, 36 individuals (16 diploids, 20 tetraploids) were assigned to three nitrogen concentrations of urea (low = 10 mg L^{-1}, medium = 200 mg L^{-1}, high = 400 mg L^{-1}) where phosphorus was kept at a constant concentration of 40 mg L^{-1} to represent the recorded phosphorus content in the field (Fig. 4.1). Five to seven individuals of each ploidy level were assigned to each concentration. Similarly, 36 different individuals (19 diploids, 17 tetraploids) were assigned to three phosphorus concentrations of superphosphate (low = 1 mg L^{-1}, medium = 20 mg L^{-1}, high = 40 mg L^{-1}) where nitrogen was kept at a constant concentration of 40 mg L^{-1} to represent an instance of low nitrogen content for this taxon (Fig. 4.1). For the NaCl treatment, 24 different individuals (12 diploids, 12 tetraploids) were assigned to two NaCl concentrations (low = 50 mM L^{-1}, high = 200 mM L^{-1}) where nitrogen, potassium, and phosphorus were excluded (Fig. 4.1). Six individuals of each ploidy were assigned to each concentration. We did not change the treatments during the experiment, so individuals received the concentration assigned to them every other day of the week for six weeks. For a control treatment, 12 different individuals received plain tap water at the same frequency where the nitrogen, phosphorus, and NaCl concentrations were excluded (Fig. 4.1).

On the day that the experiment ended, we counted the total number of leaves, measured the largest-leaf length (mm) and width (mm), and counted the number of flowers and seeds on all individuals. We calculated the relative growth rate of each individual from the beginning to the end of the experiment (over six weeks) and inspected the individuals for new stolons. Relative growth rate was calculated as plant size (product of number of leaves and largest-leaf width) difference between week one (baseline morphological trait measurements) and week six (final morphological trait measurements) of the experiment, divided by plant size at week one (e.g., Wei *et al*., 2020). Leaf length was not included in the formula to calculate plant size because previous work found that largest-leaf length did not significantly differ between diploids and tetraploids (Mtileni *et al*., 2024). We used two different, but complementary approaches, to test if diploids and tetraploids differed in the measured traits under the same treatments. First, two-way ANOVAs were used to test if the interaction of ploidy and treatment had a significant effect on the traits. Population was included as a random effect. The *lme4* and *lmerTest* packages (Bates *et al*., 2015; Kuznetsova *et al*., 2017) were used to construct the mixed models. Response variables were log-transformed to improve normality. A significant effect involving ploidy and treatment interaction would indicate that diploid and tetraploid individuals differed in their traits under the treatments. The three treatments (nitrogen, phosphorus, and NaCl) were analysed separately, and only the relative growth rate was assessed for the NaCl treatment. Second, to estimate the effect size of the response of plants to the nutrients based on traits, we calculated Hedge's *g* (Borenstein *et al*., 2011) using ploidy means for each trait (i.e., mean of each trait per ploidy) from the control treatment (control = no nutrients) and the nitrogen treatment (three concentrations pooled = 10, 200, and 400 mg L^{-1}) as well as the phosphorus treatment (three concentrations pooled = 1, 20, and 40 mg L^{-1}) as:

$$J = 1 - \frac{3}{(4df-1)},$$

$$d = \frac{Mean1\ (nutrient) - Mean2\ (control)}{\sqrt{((n1-1)S1\hat{}2+(n2-1)S2\hat{}2/(n1+n2-2))}},$$

$$g = J * d$$

where J is the correction factor and d is the standardised mean difference between two groups (control vs phosphorus and then control vs nitrogen), with S_1 and S_2 representing the standard deviations, n_1 and n_2 representing the sample sizes of the groups, and df representing the degrees of freedom ($n_1 + n_2 - 2$). We then calculated the standard error as:

$$Vg = J^2 * Vd,$$

$$Vd = \frac{n1+n2}{n1n2} + \frac{d^2}{2(n1+n2)},$$

$$SEg = \sqrt{Vg}$$

where Vd is the variance of d calculated using the formula above. After measuring the traits, we assessed the above- and belowground biomass by uprooting the individuals, rinsing them with water and then separating them one plant at a time into belowground (roots, bulb, and stolons) and aboveground (stem and leaves) biomass. We used individuals in the highest phosphorus concentration and the control treatment for this step because their flowers had already senesced, and no new buds or flowers were observed on the individuals. Individuals in the nitrogen treatment were not used because they had active flowers and new buds at this stage of the experiment. The plant samples were placed into separate paper bags, oven-dried at 37 °C for 72 hours and then weighed to the nearest gram (1 g). Kruskal-Wallis tests were used to compare the above- and belowground biomass measurements between the control treatment and highest phosphorus concentration to test if the individuals had gained biomass in response to phosphorus enrichment.

Reciprocal transplant experiment

One diploid and one tetraploid population of *R. baurii* var. *platypetala* were selected for reciprocal transplants per our permit regulation and our inability to access other populations due to bad weather conditions. The habitats of the two populations were characterized by dry, shallow stony soils over rock sheets as described by Hilliard and Burtt (1978). The diploid population (Highmoor, 1940 m a.s.l.) is north of the tetraploid population distribution (Mt Gilboa, 1750 m a.s.l.) in Kwa-Zulu Natal province, and the populations are 96.6 km away from each other. Soil analyses of the populations showed that the tetraploid population had slightly higher nitrogen, phosphorus, and potassium estimates than the diploid population (Mtileni *et al*., 2024). These two populations also differ in climatic conditions (Table S1). Individuals in the tetraploid population generally experience warmer temperatures and more infrared radiation intensity than individuals in the diploid population (Table S1; Mtileni *et al*., 2024). We originally intended to use newly germinated seeds for this experiment, but the taxon's tiny seed size and difficulty in establishing seedlings made it difficult to track the young germinants in the field. Also, young germinants did not generally survive to mature seedlings when transplanted into soil. After growing the seedlings for over six months in the growth chambers and the greenhouse, we observed that they generally only possess two-three small leaves (~1 cm long), with a very small belowground bulb (< 5 mm in diameter), rendering them unlikely to survive the experiment. Therefore, we elected to transplant adult individuals.

In November 2022, 30 individuals were collected from each population and then transplanted to the habitat of the other ploidy level until the next growing season (October–November 2023) – i.e., diploids transplanted to the tetraploid habitat, and tetraploids transplanted to the diploid habitat, after which the individuals were dug out and removed from the populations per permit stipulations. Flowers and seeds were removed from the plants before they were transplanted to remove the potential for contamination of gene pools

via pollen transfer and spread of seeds. As these plants also reproduce via underground stolons, we transplanted the individuals ~10 m away from the native individuals, in a marked ~1.5 m^2 grid for tracking so we could dig out the plants and their soil as soon as the flowering season started towards the end of October 2023 (Fig. 4.4). We counted the number of individuals that emerged as a measure of survival, noted if they were flowering, counted the number of leaves and measured leaf traits (see below) as measures of performance. We used digital calipers (GV9371, 150 mm, Grip) which were reset to zero before each measurement to measure the largest-leaf length (leaf blade tip to leaf base) and corresponding central leaf width per plant. All transplanted individuals were removed from the field after the experiment and housed in the greenhouse at the University of the Witwatersrand, South Africa. After the flowers senesced, we inspected the individuals for seeds and new stolons.

RESULTS

Field and greenhouse trait measurements

There was a significant interaction effect of ploidy and growing site on the measured traits, indicating that both polyploidy and environmental variables affected these traits (Table 4.1). Greenhouse measurements were comparable to field measurements where diploid individuals had a greater number of leaves and flowers and tetraploid individuals had consistently wider leaves than diploid individuals (Fig. 4.2). Overall, plants in the field were bigger than plants that were measured in the greenhouse, except for the length of the largest leaf where diploid individuals in the greenhouse had longer leaves than plants in the field (Fig. 4.2).

Soil conditions and plant growth

There was no significant effect of the interaction of ploidy and nutrient treatments in the measured traits, indicating that the traits did not significantly differ between diploids and

tetraploids under the nitrogen and phosphorus treatments (Table 4.2A, B). There was also no consistent trend in the traits of plants with different ploidy level under nitrogen and phosphorus (Fig. 4.3). Similarly, the effect sizes of the measured traits in response to the nitrogen and phosphorus treatments were similar for diploid and tetraploid individuals, indicating that these two treatments did not appear to affect the growth of diploids and tetraploids (Fig. 4.4). The above- and belowground biomass did not significantly differ between diploid and tetraploid individuals under the control treatment and also for the highest phosphorus concentration (40 mg L^{-1}; Table 4.3), indicating that individuals did not gain biomass in response to phosphorus enrichment.

Four seeds total were observed and collected during the experiment from two diploid individuals (n = 3 and n = 1 between the two individuals). The number of stolons did not significantly differ between diploid and tetraploid individuals across treatments (mean number of stolons for diploids = 1.32 and tetraploids = 1.05; Wilcoxon test W = 838.5*, P* = 0.83). Three tetraploid individuals did not survive under the NaCl concentrations whereas all diploid individuals exposed to saline conditions survived to the end of the experiment. The interaction of ploidy and the NaCl treatment did not influence the relative growth rate of diploid and tetraploid individuals (Table 4.2C).

Reciprocal transplant experiment

Twenty tetraploid (survivorship = 66.67 %) and 23 diploid (survivorship = 76.67 %) individuals emerged from the soil in the 2023 growing season after being reciprocally transplanted in 2022 over 11 months (Nov 2022–Oct 2023). Nine diploid individuals were flowering in October 2023 after being transplanted over the 11 month period whereas no flowers (active and/or senesced) were observed on transplanted tetraploid individuals (Fig. 4.5). Seeds and new stolons were not observed on the individuals regardless of ploidy level. Diploid individuals had more leaves and flowers than tetraploid individuals, whereas

tetraploid individuals had wider leaves (Figs. 4.5 and 4.6). Overall, diploids and tetraploids survived in each other's environment over the 11 month period.

DISCUSSION

Polyploidy affects plant phenotypes and may enhance the response of plants under different environmental conditions, sometimes conferring an advantage for polyploid plants (Porturas *et al*., 2019) and high growth and competitiveness under environmental conditions such as increased nitrogen and phosphorus (Leitch and Leitch, 2008; Faizullah *et al*., 2021). Polyploid plants have also been shown to tolerate environmental stressors such as salinity better than their diploid progenitors (Chao *et al*., 2013; Yang *et al*., 2014). In this study, we first established whether tetraploids of *R. baurii* var. *platypetala* had larger leaves and larger flowers than diploids regardless of growing site (field or greenhouse). We tested for these differences because previous work (Mtileni *et al*., 2024) found that both ploidy and localities where populations occur had an effect on leaf and flower traits such as largest-leaf width and largest-flower tepal size, and there was a significant correlation between the traits and precipitation and horizontal infrared radiation intensity. We then compared leaf and flower traits of diploid and tetraploid individuals in field populations to the same traits taken on plants that were housed in the greenhouse for over four years under the same environmental conditions as opposed to variable field conditions. Similarly to previous work, we found that diploid individuals had more leaves and flowers whereas tetraploid individuals had wider leaves regardless of where the plants grew. Further work is necessary to test whether morphological traits change in the same growing site over different years, to infer if the traits may be plastic to changing environmental conditions within a site.

We found similar morphological traits between diploids and tetraploids under nutrient enrichment treatments. Increased nitrogen and phosphorus often favour the growth of

polyploids due to their higher requirements for these nutrients compared to their diploid progenitors (Leitch and Leitch, 2008; Faizullah *et al*., 2021). In *R. baurii* var. *platypetala*, previous work found that tetraploid plants tended to occur in a habitat with slightly nutritious soils than diploids, suggesting that the ploidy levels might respond differently to nutrient enrichment. Our findings coincide with a similar experiment on *Butomus umbellatus* (Butomaceae) where polyploids did not exhibit a higher response to nutrient-rich conditions than diploids (Harms *et al*., 2021). We also found that tetraploids did not exhibit a greater number of seeds and stolons than diploids under nutrient enrichment. Tetraploids had a slightly larger belowground biomass on average than diploids under increased phosphorus concentration, but this trait did not significantly differ between diploids and tetraploids. Contrary to our findings, polyploids of *Chamerion angustifolium* exhibited greater belowground biomass in response to increased soil nitrogen and phosphorus than diploids (Onagraceae; Walczyk and Hersch-Green, 2019), suggesting that prolonged nutrient-rich conditions may ultimately favour the growth and establishment of tetraploids over diploids (Šmarda *et al*., 2013) and that tetraploids may potentially benefit from increased belowground biomass when competing for nutrients (Gioria and Osborne, 2014). It is possible that we did not run our experiment long enough to observe nutrient effects on the individuals.

The relationship between ploidy and the ecology of *Rhodohypoxis* remains unclear. In the field, *R. baurii* var. *platypetala* occurs in the alpine and montane grasslands where soils exhibit some variation in levels of electrical conductivity and exchangeable sodium within the grasslands (Nell and van Huyssteen, 2016), so we hypothesized that salinity might also influence ploidy level distribution. We included a salinity treatment in the growth chambers as a general stress to test if diploids and tetraploids responded differently under saline conditions. Three tetraploids died after being exposed to saline conditions over six weeks

whereas all diploid plants survived under the same conditions, indicating that polyploids in this taxon may not have an advantage over their progenitors under saline conditions compared to other species (Chao *et al*., 2013; Yang *et al*., 2014). The interaction of ploidy and the salinity treatment did not influence the relative growth rate of diploid and tetraploid individuals in our experiment. A possible explanation for our finding is that *R. baurii* var. *platypetala* plants do not occur in increasingly saline habitats in the Drakensberg grasslands (Nell and van Huyssteen, 2016) where salt tolerance could be beneficial.

Ecological divergence may exist for diploids and polyploids in natural environments in other plant systems (Thompson *et al*., 2014; Visger *et al*., 2016; but see Glennon *et al*., 2014) and ploidy levels may respond differently after being transplanted between their natural habitats (Ramsey, 2011). Here, we used reciprocal transplants to test whether diploid and tetraploid individuals of *R. baurii* var. *platypetala* differed in their leaf and flower traits, reproductive output (seeds and stolons), and survival in the habitat of the other ploidy level over 11 months. We found that both diploid and tetraploid individuals showed a similar survivorship in their transplant habitats, suggesting a possibility for the ploidy levels to co-exist in the field. The transplanted plants emerged from the soil around the same time in October after dying back over winter (Mar–Sept), so our results were not likely to be influenced by phenological effects. Diploid and tetraploid individuals of this taxon do not generally co-occur in the field (Mtileni *et al*., 2024), and our present findings suggest that this observed ploidy-level geographic segregation is unlikely due to potential differential response of plants with different ploidies to different environmental variables which may hinder their co-existence. We acknowledge the possibility that the ploidy levels might be already adapted to the habitats in the field (e.g., Ramsey, 2011) although the ‘away’ populations had slightly fewer or smaller traits (e.g., fewer and shorter leaves) than traits measured in ‘home’ populations. This possibility of local adaptation might be supported by

the difference in traits (e.g., number of flowers and the number and size of leaves) between field habitats and the greenhouse, where tetraploids and diploids had fewer leaves and flowers in the greenhouse and at their transplant habitats compared to their 'home' habitats. Greenhouse observations also suggest that these plants take about one year to adjust after being transplanted from the field regardless of ploidy level, which might also explain the absence of seeds or stolons on the transplanted individuals. We know the plants do not do the best (e.g., low reproductive output or fewer plants emerge from the soil after dying back over winter) in year one after a transplant, so this might have affected the results. Further reciprocal transplant work is needed to contrast trait measurements from 'home' populations with those taken in 'away' populations from plants that underwent a similar transplantation treatment. Here, local adaptation would be indicated by instances where the ploidy levels perform better at their home habitats than in the habitats of the other ploidy level (Kawecki and Ebert, 2004). Our present findings indicate that the distribution differences of diploids and tetraploids in this taxon (Mtileni *et al*., 2024) are not adaptive to the environment in general and not to the soil chemistry.

We conclude that soil nitrogen and phosphorus content did not lead to trait differences between diploids and polyploids, and that the combination of environmental factors did not affect the distribution of both ploidy levels in the field. Examining the association of increased ploidy with plant traits and potential responses of plants with different ploidies to environmental conditions provides a useful opportunity to test if polyploidy generally confers an advantage for plants under different environmental conditions, where we can generally infer how polyploidy might contribute to plant establishment and distribution. A population genetic study is also necessary to establish whether there are multiple origins of the polyploid lineages, where plants may respond differently to treatments based on their origin or unique lineage. Our present findings show that increased ploidy in *R. baurii* var. *platypetala* does not

necessarily confer an advantage under different experimental treatments and environmental conditions in the field.

ACKNOWLEDGEMENTS

We thank T. Moloi, H. Niemann, A. Theron for their assistance during this study. The study was conducted under the Eastern Cape Department of Economic Development, Environmental Affairs, and Tourism permit (CRO 131/15CR) to K.L.G. and eKZN permit (W/2180/01) to M.P.M. We thank S.L. Payne-Smith for her feedback which improved this paper. This work was supported by a National Research Foundation Grant (118526) to K.L.G. and a National Research Foundation Innovation Scholarship (131342) to M.P.M.

AUTHOR CONTRIBUTIONS

M.P.M. and K.L.G. designed the study. M.P.M. performed data collection and statistical analyses and prepared the first draft. K.L.G. provided input on subsequent drafts. All authors approved the final manuscript.

DATA AVAILABILITY

Data available at OSF link

COMPETING INTERESTS

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.

Literature Cited

Anneberg TJ, Segraves KA. 2020. Nutrient enrichment and neopolyploidy interact to increase lifetime fitness of *Arabidopsis thaliana*. *Plant and Soil* 456:439–453

Bates D, Mächler M, Bolker BM, *et al*. 2015. Fitting linear mixed- effects models using lme4. *Journal of Statistical Software* 6:1–48

Bomblies K. 2020. When everything changes at once: finding a new normal after genome duplication. *Proceedings of the Royal Society B* 287:20202154

Borenstein M, Hedges LV, Higgins JPT, *et al*. 2011. Introduction to meta-analysis. Chichester, West Sussex, United Kingdom: John Wiley & Sons

Domínguez-Delgado JJ, López-Jurado J, Mateos-Naranjo E, *et al*. 2021. Phenotypic diploidization in plant functional traits uncovered by synthetic neopolyploids in *Dianthus broteri*. *Journal of Experimental Botany* 72:5522–5533

Chao DY, Dilkes B, Luo H, *et al*. 2013. Polyploids exhibit higher potassium uptake and salinity tolerance in *Arabidopsis*. *Science* 341:658–659

Doležel J, Greilhuber J, Suda J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols* 2:2233–2244

Doyle JJ, Coate JE. 2019. Polyploidy, the nucleotype, and novelty: the impact of genome doubling on the biology of the cell. *International Journal of Plant Sciences* 180:1–52

Faizullah L, Morton JA, Hersch-Green EI, *et al*. 2021. Exploring environmental selection on genome size in angiosperms. *Trends in Plant Science* 26:1039–1049

Gioria M, Osborne BA. 2014. Resource competition in plant invasions: emerging patterns and research needs. *Frontiers in Plant Science* 5:501

Glennon KL, Ritchie ME, Segraves KA. 2014. Evidence for shared broad-scale climatic niches of diploid and polyploid plants. *Ecology Letters* 17:574–582

Guan Z, Zhang S, Guan K, *et al*. 2011. Leaf anatomical structures of *Paphiopedilum* and *Cypripedium* and their adaptive significance. *Journal of Plant Research* 124:289– 298

Guignard MS, Nichols RA, Knell RJ, *et al*. 2016. Genome size and ploidy influence angiosperm species' biomass under nitrogen and phosphorus limitation. *New Phytologist* 210:1195–1206

Harms NE, Cronin JT, Gaskin JF. 2021. Increased ploidy of *Butomus umbellatus* in introduced populations is not associated with higher phenotypic plasticity to N and P. *AoB PLANTS* 13:plab045

Hilliard OM, Burtt BT. 1978. Notes on some plants from southern Africa chiefly from Natal. VII. Notes from the Royal Botanic Garden, Edinburgh 36:43–76

Husband BC, Baldwin SJ, Suda J. 2013. The incidence of polyploidy in natural plant populations: major patterns and evolutionary processes. *Plant Genome Diversity Volume 2*, I.J. Leitch et al. (eds.), 255–276. Springer Vienna

Kawecki TJ, Ebert D. 2004. Conceptual issues in local adaptation. *Ecology letters* 7:1225–1241

Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software* 82:1–26

Leitch AR, Leitch IJ. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* 320:481–483

Liu S, Chen S, Chen Y, *et al*. 2011. In vitro induced tetraploid of *Dendranthema nankingense* (Nakai). Tzvel. shows an improved level of abiotic stress tolerance. *Scientia Horticulturae* 127:411–419

Maherali H, Walden AE, Husband BC. 2009. Genome duplication and the evolution of physiological responses to water stress. *New Phytologist* 184:721–731

Masterson J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science* 264:421–424

Marchant DB, Soltis DE, Soltis, PS. 2016. Patterns of abiotic niche shifts in allopolyploids relative to their progenitors. *New Phytologist* 212:708–718

Mtileni MP, Venter N, Glennon KL. 2021. Ploidy differences affect leaf functional traits, but not water stress responses in a mountain endemic plant population. *South African Journal of Botany* 138:76–83

Mtileni MP, Le Maitre NC, Steenhuisen S. *et al.* 2024. Increased solar radiation and soil moisture determine flower colour frequency in a mountain endemic plant population. *Plant Ecol*ogy 225:201–211

Mtileni MP, Oberlander KC, Glennon KL. 2024. Morphological and habitat differentiation between diploids and tetraploids of a Drakensberg near-endemic taxon, *Rhodohypoxis baurii* var. *platypetala* (Hypoxidaceae). *Austral Ecology* 49:13502

Nell JP, van Huyssteen CW. 2016. Quantification of primary salinity, sodicity and alkalinity in South African soils using climate regions. *South African Journal of Plant and Soil* 33:289–296

Otto SP, Whitton J. 2000. Polyploid incidence and evolution. *Annual Review of Genetics* 34:401–437

Picotte JJ, Rosenthal DM, Rhode JM, *et al*. 2007. Plastic responses to temporal variation in moisture availability: consequences for water use efficiency and plant performance. *Oecologia* 153:821–832

Porturas LD, Anneberg TJ, Curé AE, *et al*. 2019. A meta-analysis of whole genome duplication and the effects on flowering traits in plants. *American Journal of Botany* 106:469–476

Ramsey J. 2011. Polyploidy and ecological adaptation in wild yarrow. *Proceedings of the National Academy of Sciences of the United States of America* 108:7096–7101

Ramsey J, Ramsey TS. 2014. Ecological studies of polyploidy in the 100 years following its discovery. *Philosophical Transactions of the Royal Society B* 369:20130352

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Saito K. 1975. Studies on the occurrence of polyploidy and its contributions to the flower plant breeding. XII. On the nature and sterility in the spontaneous triploid cultivars of *Rhodohypoxis baurii* Nel. *Japanese Journal of Plant Breeding* 25:355–362

Šmarda P, Hejcman M, Březinová A, *et al*. 2013. Effect of phosphorus availability on the selection of species with different ploidy levels and genome sizes in a long-term grassland fertilization experiment. *New Phytologist* 200:911–921

Walczyk AM, Hersch-Green EI. 2019. Impacts of soil nitrogen and phosphorus levels on cytotype performance of the circumboreal herb *Chamerion angustifolium*: implications for polyploid establishment. *American Journal of Botany* 106:906–921

Wei N, Cronn R, Liston A, *et al*. 2019. Functional trait divergence and trait plasticity confer advantage in heterogenous environments. *New Phytologist* 221:2286–2297

Wei N, Du Z, Liston A, *et al*. 2020. Genome duplication effects on functional traits and fitness are genetic context and species dependent: studies of synthetic polyploid *Fragaria*. *American Journal of Botany* 107:262–272

te Beest M, Le Roux JJ, Richardson DM, *et al*. 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* 109:19–45

Thompson KA, Husband BC, Maherali H. 2014. Climatic niche differences between diploid and tetraploid cytotypes of *Chamerion angustifolium* (Onagraceae). *American Journal of Botany* 101:1868–1875

Visger CJ, Germain-Aubrey CC, Patel M, *et al*. 2016. Niche divergence between diploid and autotetraploid *Tolmiea*. *American Journal of Botany* 103:1396–1406

Volkmar KM, Hu Y, Steppuhn H. 1998. Physiological responses of plants to salinity: A review. *Canadian Journal of Plant Science* 78:19–27

Yang C, Zhao L, Zhang H, *et al*. 2014. Evolution of physiological responses to salt stress in hexaploid wheat. *Proceedings of the National Academy of Sciences of the United States of America* 111:11882–11887

Yu F, Kress WJ, Gao J-Y. 2010. Morphology, distribution, and chromosome counts of two varieties of *Hedychium villosum* (Zingiberaceae). *Journal of Systematics and Evolution* 48:344–349

Zhang XY, Hu CG, Yao JL. 2010. Tetraploidization of diploid *Dioscorea* results in activation of the antioxidant defense system and increased heat tolerance. *Journal of Plant Physiology* 167:88–94

Table 4.1 Summary results from Type III ANOVA analyses of the interaction of ploidy and growing site (field or greenhouse) and their effect on the number of leaves and flowers, largest-leaf length (mm) and width (mm) of *Rhodohypoxis baurii* var. *platypetala* diploid and tetraploid individuals. Significant results at $P = 0.05$ are bolded and 0.0001 represents values that are extremely small. The *lme4* and *lmerTest* packages were used to construct the models.

	Ploidy				Site				Ploidy x Site			
Trait	Sum Squares	df	*F* value	*P*	Sum Squares	df	*F* value	*P*	Sum Squares	df	*F* value	*P*
Number of leaves	0.60	1	10.57	**0.02**	1.86	1	32.55	**0.0001**	0.35	1	6.09	**0.01**
Number of flowers	0.28	1	1.80	0.24	14.88	1	97.46	**0.0001**	1.07	1	6.98	**0.01**
Largest-leaf length (mm)	0.61	1	3.52	0.12	1.36	1	7.78	**0.01**	1.72	1	9.83	**0.002**
Largest-leaf width (mm)	0.36	1	4.29	0.12	4.95	1	59.54	**0.0001**	2.80	1	33.67	**0.0001**

Table 4.2 Summary results from Type III ANOVA analyses of the interaction of ploidy and experimental treatments [(nitrogen (A), phosphorus (B) and NaCl (C)] and their effect on the number of leaves and flowers, largest-leaf length (mm) and width (mm), relative growth rate and the number of stolons of *Rhodohypoxis baurii* var. *platypetala* diploid and tetraploid individuals. Significant results at $P = 0.05$ are bolded and 0.0001 represents values that are extremely small. The *lme4* and *lmerTest* packages were used to construct the models.

(A) Nitrogen

	Ploidy				Treatment				Ploidy x Treatment			
Trait	Sum Squares	df	*F* value	*P*	Sum Squares	df	*F* value	*P*	Sum Squares	df	*F* value	*P*
Number of leaves	1.24	1	9.40	**0.01**	0.71	3	1.79	0.16	0.08	3	0.21	0.89
Number of flowers	0.96	1	3.29	0.08	0.36	3	0.41	0.75	0.36	3	0.42	0.74
Largest-leaf length (mm)	0.99	1	7.83	**0.02**	0.49	3	1.28	0.29	0.15	3	0.40	0.75
Largest-leaf width (mm)	3.02	1	31.05	**0.0001**	0.15	3	0.50	0.69	0.12	3	0.41	0.74
Relative growth rate	0.03	1	0.10	0.75	1.36	3	1.51	0.23	0.26	3	0.29	0.83
Number of stolons	0.0003	1	0.0006	0.98	1.08	3	0.77	0.52	0.39	3	0.28	0.84

(B) Phosphorus

	Ploidy				Treatment				Ploidy x Treatment			
Trait	Sum Squares	df	*F* value	*P*	Sum Squares	df	*F* value	*P*	Sum Squares	df	*F* value	*P*
Number of leaves	0.51	1	5.79	0.05	0.21	3	0.79	0.51	0.10	3	0.38	0.77
Number of flowers	0.20	1	0.87	0.37	2.84	3	4.14	**0.01**	1.001	3	1.46	0.24
Largest-leaf length (mm)	0.31	1	2.50	0.14	2.60	3	6.92	**0.001**	0.85	3	2.27	0.10
Largest-leaf width (mm)	0.48	1	8.28	**0.02**	0.36	3	2.05	0.12	0.11	3	0.62	0.61
Relative growth rate	0.46	1	2.73	0.20	1.00	3	1.97	0.13	0.20	3	0.41	0.75
Number of stolons	0.001	1	0.003	0.96	0.12	3	0.11	0.95	1.07	3	0.97	0.42

(C) NaCl

	Ploidy				Treatment				Ploidy x Treatment			
Trait	Sum Squares	df	*F* value	*P*	Sum Squares	df	*F* value	*P*	Sum Squares	df	*F* value	*P*
Relative growth rate	0.18	1	0.52	0.49	13.21	2	18.77	**0.0001**	0.33	2	0.49	0.63

Table 4.3 Mean ± SE of above- and belowground biomass (g) of diploid and tetraploid individuals of *Rhodohypoxis baurii* var. *platypetala* under the control treatment and the experimental 40 mg L^{-1} phosphorus concentration. There was no significant difference between the treatments ($P > 0.05$) for aboveground biomass ($P = 0.87$) and belowground biomass ($P = 0.92$) following Kruskal-Wallis tests.

		Aboveground biomass (g)	Belowground biomass (g)
Ploidy	Treatment	Mean ±SE	Mean ±SE
Diploid	Control	0.05 ± 0.01	0.15 ± 0.05
Tetraploid	Control	0.06 ± 0.03	0.75 ± 0.15
Diploid	High P	0.07 ± 0.0 2	0.66 ± 0.49
Tetraploid	High P	0.05 ± 0.02	1.75 ± 1.27

Figure Legend

Fig. 4.1 The design of the growth chamber experiment that combines three treatments of phosphorus (P), nitrogen (N) and salinity (NACl) to test the effect of these treatments on the growth of *Rhodohypoxis baurii* var. *platypetala* diploid (2*x*) and tetraploids (4*x*) plants. The N and P treatments comprised three concentrations, and the NACl treatment comprised two concentrations. The number of plants assigned to each concentration per ploidy level are shown in parentheses.

Fig. 4.2 Mean ± standard error (SE) for traits of *Rhodohypoxis baurii* var. *platypetala* diploid (grey) and tetraploid (black) individuals measured in the greenhouse and also in field populations. Measured traits were the number of leaves (A) and flowers (B), width (C) and length (D; mm) of the largest leaves per plant. Asterisks indicate significant results at $P = 0.0001$*** and $P = 0.001$** following Kruskal-Wallis tests. Trait comparisons between diploids and tetraploids were done separately for the field and greenhouse data.

Fig. 4.3 Mean ± SE for traits of *Rhodohypoxis baurii* var. *platypetala* diploid (grey) and tetraploid (black) individuals under nitrogen or phosphorus enrichment. The connected lines do not indicate a temporal connection between the treatments, but that diploid and tetraploid individuals were very similar in traits in response to the treatments.

Fig. 4.4 Hedge's *g* ± SE of *Rhodohypoxis baurii* var. *platypetala* diploid (grey) and tetraploid (black) individuals in response to nitrogen or phosphorus enrichment. The black dashed line represents a small effect size benchmark of 0.2. Moderate and large effect sizes are not indicated on the figure because we did not Hedge's *g* estimates greater than 0.2 (small effect size). Traits measured were the number of stolons (S), number of leaves (L) and flowers (F), largest-leaf length (LL) and width (LW), and the relative growth rate (RGR). Zero represents a benchmark value to indicate negative (<0) and positive (>0) effect sizes.

Fig. 4.5 Photographs of experimental transplants using *Rhodohypoxis baurii* var. *platypetala*. Tetraploid individuals were transplanted to a diploid habitat in the field (A; three arrows) and diploid individuals to a tetraploid habitat in the field (B; five arrows). After 11 months, we compared the survivorship of the tetraploid (C) and diploid (D) individuals.

Fig. 4.6 Measurements of *Rhodohypoxis baurii* var. *platypetala* diploid (2x) and tetraploid (4x) individuals based on three traits: number of leaves, and largest-leaf length (mm) and width (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 outliers which are represented by black dots. Asterisks indicate significant trait differences at $P = 0.0001$ following Kruskal-Wallis tests. 'H' represents home populations of the ploidy levels and 'A' represents the habitat of the other ploidy level. We used a white colour for tetraploids because it did not conceal the medians.

Control (tap water excluding NPK + NACl)



2x (6)
4x (6)

Trt 1: Nitrogen

P (40 mg/L) + K (250 mg/L) + **N**

N concentrations:

Low (10 mg/L)	Medium (200 mg/L)	High (400 mg/L)
 2x (6) 4x (6)	 2x (6) 4x (6)	 2x (6) 4x (6)

Trt 2: Phosphorus

N (40 mg/L) + K (250 mg/L) + **P**

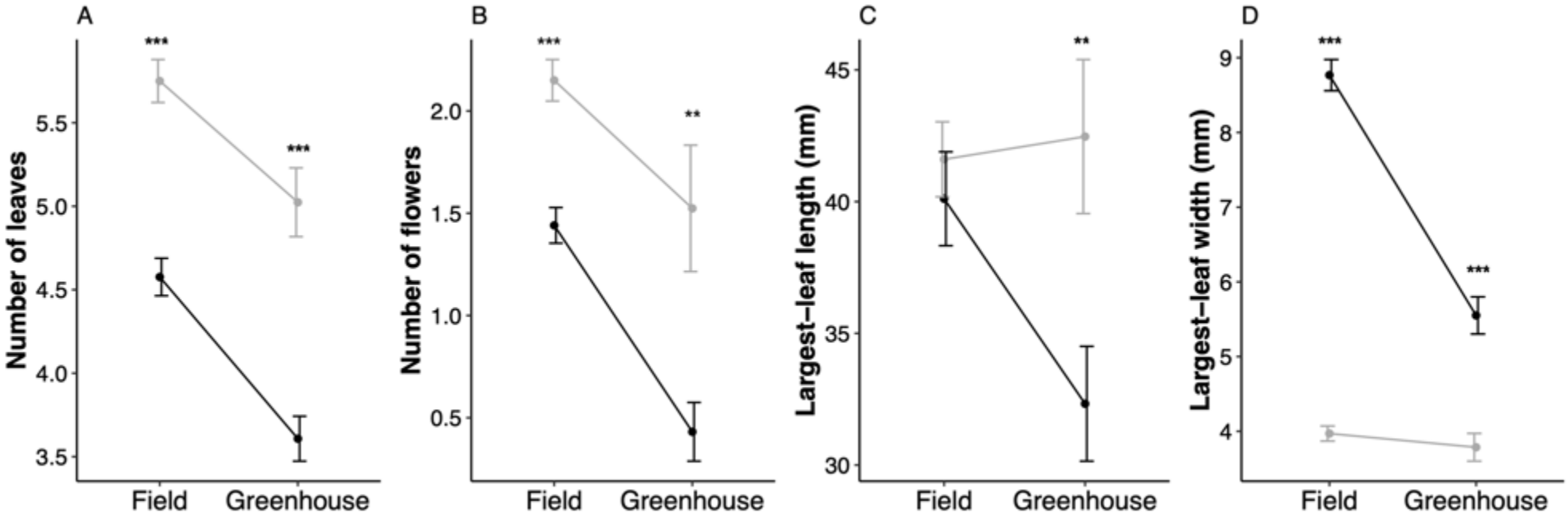
P concentrations:

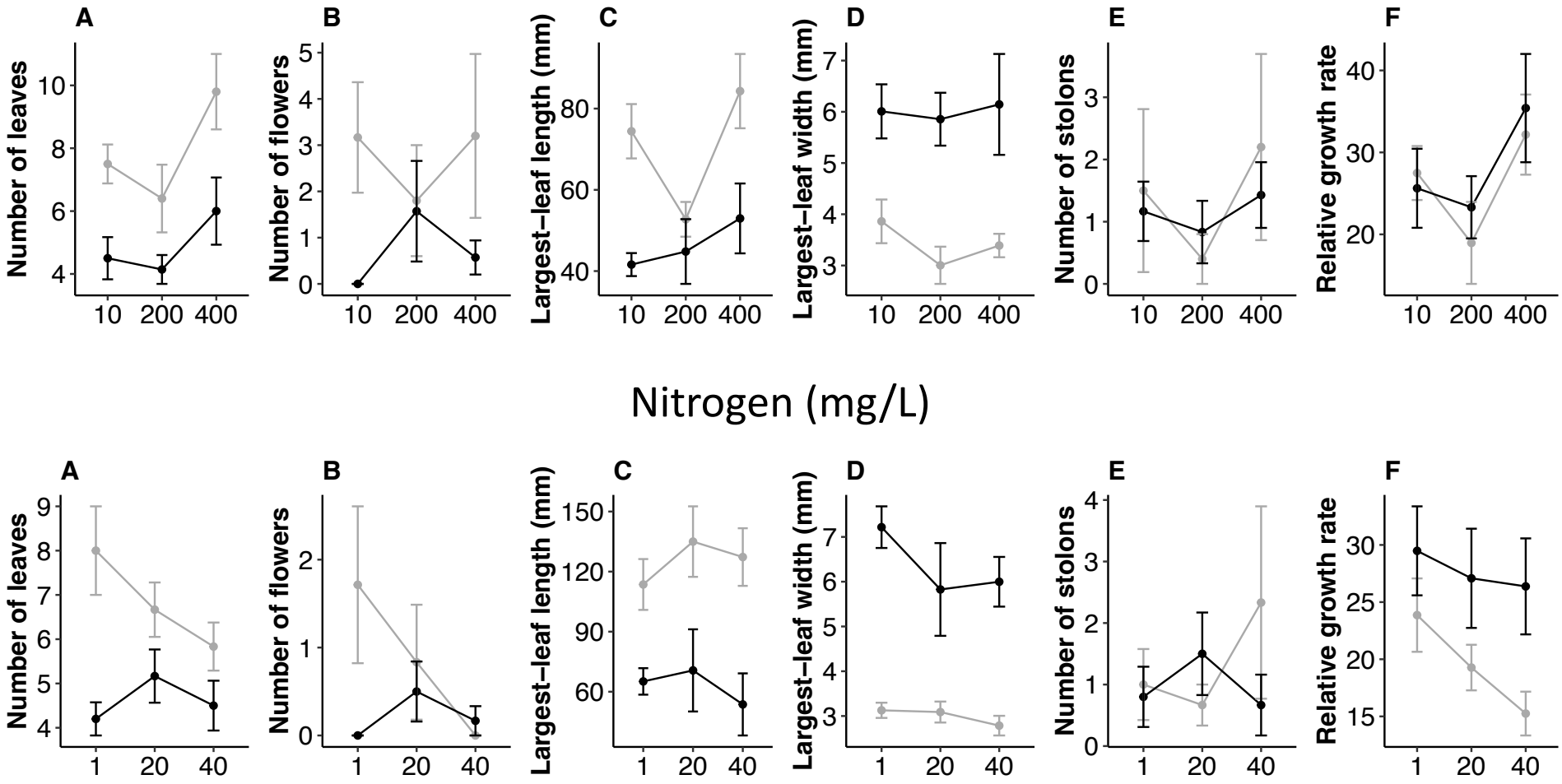
Low (1 mg/L)	Medium (20 mg/L)	High (40 mg/L)
 2x (6) 4x (6)	 2x (6) 4x (6)	 2x (6) 4x (6)

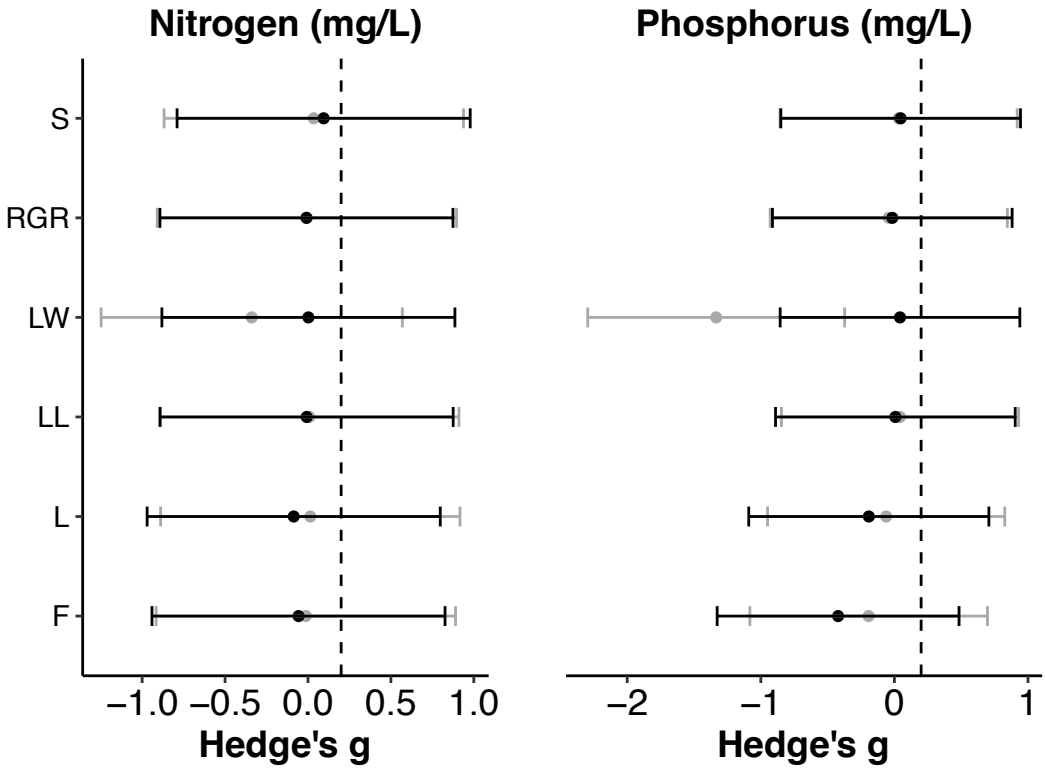
Trt 3: Salinity (excluding NPK)

NACl concentrations:

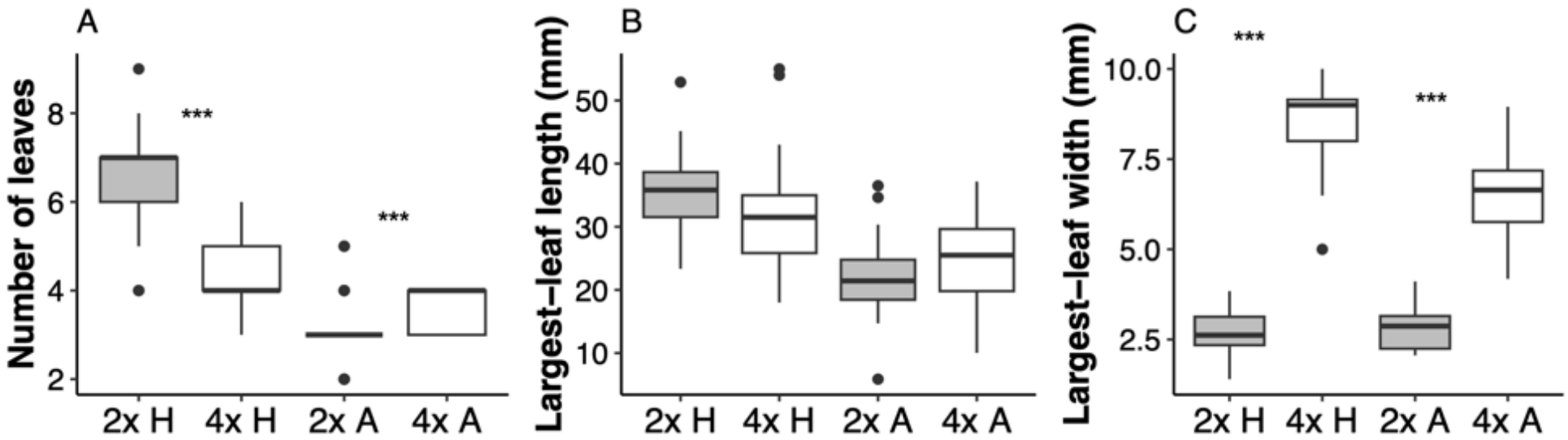
Low (50 mM/L)	High (200 mM/L)
 2x (6) 4x (6)	 2x (6) 4x (6)


A
B
C
D
Number of leaves
Number of flowers
Largest−leaf length (mm)
Largest−leaf width (mm)
Field
Greenhouse
***
**


A
Number of leaves
10
8
6
4
10 200 400
B
Number of flowers
5
4
3
2
1
0
10 200 400
C
Largest−leaf length (mm)
80
60
40
10 200 400
D
Largest−leaf width (mm)
7
6
5
4
3
10 200 400
E
Number of stolons
3
2
1
0
10 200 400
F
Relative growth rate
40
30
20
10 200 400
Nitrogen (mg/L)
A
Number of leaves
9
8
7
6
5
4
1 20 40
B
Number of flowers
2
1
0
1 20 40
C
Largest−leaf length (mm)
150
120
90
60
1 20 40
D
Largest−leaf width (mm)
7
6
5
4
3
1 20 40
E
Number of stolons
4
3
2
1
0
1 20 40
F
Relative growth rate
30
25
20
15
1 20 40
Phosphorus (mg/L)


Nitrogen (mg/L)
Phosphorus (mg/L)
S
RGR
LW
LL
L
F
−1.0 −0.5 0.0 0.5 1.0
−2 −1 0 1
Hedge's g
Hedge's g


A
B
C
D


A
Number of leaves
8
6
4
2
***
***
2x H
4x H
2x A
4x A
B
Largest−leaf length (mm)
50
40
30
20
10
2x H
4x H
2x A
4x A
C
Largest−leaf width (mm)
10.0
7.5
5.0
2.5
***
***
2x H
4x H
2x A
4x A

SUPPLEMENTARY INFORMATION

Table S1 Mean and SE of 13 climatic variables compared between the Highmoor and Mt Gilboa populations of *Rhodohypoxis baurii* var. *platypetala* that were selected for the reciprocal transplant experiment.

	Highmoor (2*x*)		Mt Gilboa (4*x*)	
Variable	Mean	SE	Mean	SE
Temperature at 2 m (ºC)	11.18	0.08	15.49	0.08
Dew/Frost Point at 2 m (ºC)	2.73	0.11	7.81	0.10
Wet Bulb Temperature at 2 m (ºC)	6.96	0.09	11.65	0.08
Earth Skin Temperature (ºC)	11.42	0.09	15.61	0.08
Temperature at 2 m maximum (ºC)	19.18	0.08	23.71	0.09
Temperature at 2 m minimum (ºC)	4.78	0.08	9.32	0.07
Longwave downward irradiance (W/m^2)	310.50	0.70	345.06	0.65
Specific humidity at 2 m (g/kg)	6.57	0.05	8.31	0.05
Relative humidity at 2 m (%)	62.90	0.25	66.77	0.25
Precipitation corrected (mm/day)	2.82	0.09	2.90	0.09
Surface soil wetness (0–1)	0.64	0.002	0.58	0.002
Root zone soil wetness (0–1)	0.66	0.001	0.59	0.001
Profile soil moisture (0–1)	0.65	0.001	0.60	0.001

CHAPTER 5

GENERAL CONCLUSION

Examining how polyploid populations establish is important to contextualise the observed differences between polyploids and their diploid progenitors, such as morphological and habitat differentiation. In this thesis, I investigated how environmental variables shape the distribution of different ploidy levels of plant species and populations. Findings from this thesis corroborate results from previous large scale studies which showed that the association of polyploid occurrence with climate variables, elevation, and soil moisture remains unresolved. Generally, the meta-analysis within this thesis showed that there is limited evidence for polyploids occurring at higher elevations than diploids. Overall, elevation, climate variables, and soil moisture explained a tiny variance of polyploid distribution. While large-scale work provides important insights for understanding the general contribution of polyploidy to plant ecology and also reveals emergent patterns that may not be evident at small scales, broad scale results are unlikely representative of all plant systems and populations.

Scale is important for understanding how polyploid plants are distributed relative to their diploid progenitors. Moreover, comparing environmental niches of diploid and polyploid populations from the same species is useful to refine the biogeographic patterns of polyploid plants than collectively analysing polyploid species across the globe. Indeed, my review of cytogeographic studies of many mixed-ploidy species showed discordance in niches between diploids and polyploids, where niche divergence and niche overlap were found in different studies and there was no clear pattern of polyploid plants occurring at higher elevations. If niche divergence is found between diploids and polyploids, experimental studies often show that diploid and polyploid plants perform equally in traits and responses under different environmental conditions (e.g., similar water use efficiency under water stress; Mtileni *et al*., 2021). Experimental studies at local scales (e.g., reciprocal transplant experiments) are useful to complement cytogeographic studies and to test the contribution of

polyploidy to plant establishment and subsequent persistence. Field work at the population level done within this thesis on the Drakensberg near-endemic taxon, *Rhodohypoxis baurii* var. *platypetala*, showed that there was habitat and morphological differentiation between diploid and polyploid plants, where tetraploids were larger and found at lower elevations, in warmer climate with drier, and in slightly more nutrient-rich soils. Field studies and experimental work on this taxon also showed that polyploidy does not necessarily confer an advantage or limitation in different conditions, a trend that has been observed in previous work in other systems (Wei *et al*., 2019, 2020; Mtileni *et al*., 2021; Harms *et al*., 2021). The habitat differentiation between diploids and polyploids of *R. baurii* var. *platypetala* does not appear to be based on the adaptation of the ploidies to their habitats.

The broader meta-analysis demonstrated that climate variables, soil moisture, and geographic region only explained 0.3% of polyploid presence at high elevations across studies. I hypothesised that there would be a trend across different studies where polyploids occur at higher elevations than diploids. I expected that this trend could be explained by the beneficial outcomes of polyploidy in plants such as increased genetic variation and greater adaptive potential to tolerate environmental conditions such as cold treatments (Leitch and Leitch, 2008; Husband *et al*., 2013). Cold environments have also been associated with the production of unreduced gametes, which primarily contribute to polyploid formation (Ramsey and Ramsey, 1998; Van de Peer et *al*., 2017). These two components might enable polyploids to be more abundant at higher elevations and latitudes. Instead, results suggested that polyploids were generally found at lower elevations than their diploid progenitors and that soil moisture, relative humidity, and temperature explained a tiny fraction of polyploid distribution at high elevations. Specifically, in *R. baurii* var. *platypetala*, the effect size of elevation on polyploid distribution was negative (Hedge's g = -0.94, Chapter 3), highlighting that tetraploids were not found at higher elevations than diploids, and that there was an

overlap in the elevation of diploid and polyploid populations. Overall, this weak general association of polyploid distribution with elevation and environmental variables (low soil moisture, high temperature range, high relative humidity) where polyploid presence was more likely supports previous work where shared broad niches were found for diploid and polyploid plants (Glennon *et al*., 2014; Marchant *el al*., 2016) or where an indirect effect of climate, soil variables, and elevation on global polyploid distribution was found (Rice *et al*., 2019; Bureš *et al*., 2024).

Going forward, a unified approach needs to be developed for modelling distributions and identifying predictor variables that focus on identifying comparable patterns of niche shifts across studies, like niche contraction and niche novelty between diploids and polyploids (e.g., Parisod and Broennimann, 2016; Marchant *et al*., 2016). It is also necessary to go beyond climate and soil in such analyses and include niche availability, species competition, and life history, which can play critical roles in polyploid distribution (e.g., Rice *et al*., 2019).

In *Rhodohypoxis*, polyploidy was not found to confer an advantage or limitation under experimental nutrient and saline treatments. Polyploid plants are thought to exhibit an increased adaptive potential or competitiveness across different environments (for review, see te Beest *et al*., 2012). Here, I found that diploid and tetraploid populations did not co-occur across the sampled range, which suggests that there are different environmental factors affecting ploidy level distribution. Indeed, tetraploid populations generally occurred in warmer and drier environments and in a habitat with marginally higher phosphorus and nitrogen than diploids, but there was overlap in precipitation, temperature, infrared radiation intensity, and soil moisture conditions between diploid and polyploid populations. I also found that leaves and flowers of tetraploid individuals were larger than diploids, except where largest-flower size was positively correlated to both ploidy and precipitation as well as

infrared radiation intensity (bigger flowers in polyploids with more rain and heat). In the growth chamber experiments, increased ploidy was not associated with larger morphological traits (e.g., increased belowground biomass) or higher reproductive output (greater number of seeds and stolons) under nutrient enrichment, nor increased survivorship under experimental saline conditions which were included in the experiment as a general stress. Previous work on this taxon also found that tetraploid individuals did not show higher water use efficiency than diploid individuals under experimental water stress (Mtileni *et al*., 2021). Together, findings from this thesis coincide with previous work where polyploidy was not found to confer an advantage or limitation under different environmental conditions such as increased nitrogen and phosphorus or water stress in different plant systems (e.g., Wei *et al*., 2019, 2020; Mtileni *et al*., 2021; Harms *et al*., 2021). This trend indicates that polyploidy leads to variable outcomes across plant lineages and that the association of polyploidy with enhanced plant responses to different environmental conditions is unlikely to be consistent.

Understanding the geographic and habitat barriers between diploids and polyploids provides invaluable information regarding polyploid establishment and distribution. The distribution pattern of diploids and tetraploids of *R. baurii* var. *platypetala* is potentially caused by a differential survival or a reproductive success (potentially local adaptation). As a result, the survival rate and reproductive output of diploids and tetraploids should differ between the ploidies if they are reciprocally transplanted between their field habitats. These plants die back over winter, so I hypothesized that individuals would need to survive a winter to be fully 'established' after being transplanted. I found that both the survival rate and reproductive output of transplanted diploid and tetraploid plants were similar, which suggests that diploids and tetraploids can establish in each other's environments. As both ploidies lived in each other's habitat, although tetraploids were not flowering when the experiment ended, this finding also suggests that collective habitats selection pressures of the diploid and

polyploid populations do not necessarily explain where different ploidies can occur. This finding supports our findings that ploidy level did not enhance the response of plants under different experimental environmental conditions.

One explanation for this pattern could be that there is hybridization among varieties and between species within the genus. This is because mating opportunities may differ between populations, which produces plants that are polyploids due to hybridization. Consequently, diploids may have been the minority ploidy level over time and subsequently died out. For instance, hybrids in other genera can live in intermediate or novel environments (e.g., *Helianthus* hybrids, Rieseberg *et al*., 2007). *Rhodohypoxis* is known to naturally hybridize with *Hypoxis*, especially *H. parvula* (Hilliard and Burtt, 1978), so further work is necessary to examine the mode of tetraploid inheritance in *R. baurii* var. *platypetala* as the type of polyploidy (allo/autopolyploidy) can affect the genetic diversity of individuals and how traits evolve.

In conclusion, the association of polyploidy with plant distribution and potential ploidy responses to environmental variables is likely to be scale-dependent, species-specific, and potentially even population-specific. We are unlikely to observe a unilateral prediction of an observed biogeographic pattern for polyploid species as patterns may also be a consequence of historical contingency and current habitats that will differ for independent plant systems. Evidence from cytogeographic studies of many mixed-ploidy species has shown support for both niche overlap and niche divergence between diploids and polyploids. Findings from this thesis contribute to the view that general comparisons of the environmental distribution of polyploid species may be confounded by factors such as different geographic regions where independent plant systems occur, climatic and soil variables that affect different populations where different ploidies occur, or the availability of space and mating opportunities, which should be clearly accounted for in studies. While the

association of polyploid plants with latitude is a striking pattern (polyploids found in colder regions), evidence for polyploids being distributed at higher elevations is limited.

A broad-scale relationship between polyploidy and environmental variables presents challenges for outlining a unifying hypothesis to explain global polyploid biogeography and potentially increasing prediction errors due to broad generalizations across plant systems, especially as the implications of polyploidy in plants are likely species-specific and unlikely to be uniform across plant systems. Work at smaller scales may collectively reveal the effects of environmental variables that affect different populations where different ploidies occur. Here, work in *R. baurii* var. *platypetala* demonstrated that the cytogeographic differentiation does not seem to be based on the adaptation of diploids and tetraploids to their habitats, which suggests that the association of polyploidy and environmental variables may be indirect. This finding also highlights a trend in polyploid research where polyploidy does not always confer an advantage or limitation for plants when living in some environmental conditions. Taken together, findings from this thesis underscore the importance of further work to examine the establishment and persistence of polyploids in shaping plant distribution as well as the direct relationship between polyploidy and their initially experienced environmental variables.

Literature Cited

Bureš P, Elliott TL, Veselý P, *et al*. 2024. The global biogeography of angiosperm genome size is shaped by climate and range size. *New Phytologist* 242:744–759

Glennon KL, Ritchie ME, Segraves KA. 2014. Evidence for shared broad-scale climatic niches of diploid and polyploid plants. *Ecology Letters* 17:574–582

Harms NE, Cronin JT, Gaskin JF. 2021. Increased ploidy of *Butomus umbellatus* in introduced populations is not associated with higher phenotypic plasticity to N and P. *AoB PLANTS* 13:plab045

Hilliard OM, Burtt BT. 1978. Notes on some plants from southern Africa chiefly from Natal. VII. Notes from the Royal Botanic Garden, Edinburgh 36:43–76

Husband BC, Baldwin SJ, Suda J. 2013. The incidence of polyploidy in natural plant populations: major patterns and evolutionary processes. *Plant Genome Diversity Volume 2*, I.J. Leitch et al. (eds.), 255–276. Springer Vienna

Leitch AR, Leitch IJ. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* 320:481–483

Marchant DB, Soltis DE, Soltis, PS. 2016. Patterns of abiotic niche shifts in allopolyploids relative to their progenitors. *New Phytologist* 212:708–718

Mtileni MP, Venter N, Glennon KL. 2021. Ploidy differences affect leaf functional traits, but not water stress responses in a mountain endemic plant population. *South African Journal of Botany* 138:76–83

Parisod C, Broennimann O. 2016. Towards unified hypotheses of the impact of polyploidy on ecological niches. *New Phytologist* 212:540–542

Ramsey J, Schemske DW. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29:467–501

Rice A, Šmarda P, Novosolov M, *et al*. 2019. The global biogeography of polyploid plants. *Nature Ecology & Evolution* 3:265–273

Rieseberg LH, Kim SC, Randell RA, *et al*. 2007. Hybridization and the colonization of novel habitats by annual sunflowers. *Genetica* 129:149–165

te Beest M, Le Roux JJ, Richardson DM, *et al*. 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* 109:19–45

Van de Peer Y, Mizrachi E, Marchal K. 2017. The evolutionary significance of polyploidy. *Nature Reviews Genetics* 18:411–424

Wei N, Cronn R, Liston A, *et al*. 2019. Functional trait divergence and trait plasticity confer advantage in heterogenous environments. *New Phytologist* 221:2286–2297

Wei N, Du Z, Liston A, *et al*. 2020. Genome duplication effects on functional traits and fitness are genetic context and species dependent: studies of synthetic polyploid *Fragaria*. *American Journal of Botany* 107:262–272