Functional traits and soil water availability shape competitive interactions in a diploid-polyploid complex

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Summary

- Even within those polyploid plant species that become established initially, only a few persist in the long term. Competitive interactions between polyploids and their diploid progenitors in secondary contact zones can contribute to local extinctions. Environmental factors such as water availability and functional trait divergence may influence these interactions.
- We conducted a greenhouse competition experiment with four cytotypes (2x, 4x, 6x, and 12x) of *Dianthus broteri* under two contrasting water regimes. We estimated niche and fitness differences and predicted the pairwise competitive outcomes. Additionally, we explored the influence of morphological and physiological functional traits (SLA, A_N, g_s, LDMC, F_v/F_m, and $_i$WUE) on competitive interactions.
- Soil water availability modified the competitive dynamics between cytotypes and predicted competitive exclusion. Under high water availability, lower ploidy levels (2x and especially 4x) outcompeted higher ploidy cytotypes (12x, 6x), while the latter exhibited greater competitive abilities under low water availability. These differences were explained by functional traits related to competitive effects (SLA) and competition tolerance (A_N, F_v/F_m, and $_i$WUE).
- Our study emphasizes that the long-term fate of polyploids largely depends on water availability, with high polyploids having a competitive advantage in arid environments. This ultimately highlights the role of functional traits in shaping the competitive dynamics between cytotypes.

Keywords: Coexistence, Competition, *Dianthus broteri*, Functional traits, Polyploidy, Water availability.

Introduction

Polyploidy is a crucial mechanism in plant evolution (Grant, 1981; Soltis et al., 2009). Despite its significant role and the high prevalence of polyploids in nature, neopolyploids face challenges in establishing themselves (Fowler & Levin, 1984; Köhler et al., 2010). Neopolyploids are subjected to strong positive frequency-dependent selection, termed "minority cytotype exclusion", as they compete with locally adapted parental diploids (Levin, 1975). Overcoming this handicap, which determines the establishment and persistence of neopolyploids, requires mechanisms such as exploiting competitive advantages or occupying unfilled ecological niches (Hijmans et al., 2007; López-Jurado et al., 2019; Ståhlberg, 2009). In addition, previous studies have shown that newly formed polyploid species experience higher extinction rates than their diploid counterparts (Mayrose et al., 2011). This indicates that even after short-term establishment, only a few polyploids persist in the long term (Arrigo & Barker, 2012) normally due to their small population sizes and restricted distributions (Anneberg et al., 2023; Van De Peer et al., 2017). In addition, another possible explanation for this low long-term success of polyploids is the interaction with their ancestral cytotype in zones of emerging secondary contact (Mortier et al., 2024), where previously separated, differentiated polyploids are brought into contact with diploid cytotypes (Mráz et al., 2012; Singliarova et al., 2019). Once in contact, the coexistence of polyploids and diploids are influenced by their different adaptive potentials and competitive interactions (Kolář et al., 2017). In this context, modern coexistence theory (MCT), a framework used for predicting plant species coexistence (Chesson, 2000), have been also applied to examine competition across different environmental conditions (Spaak et al., 2021). MCT can provide insights into the establishment process in the early stages of polyploid evolution (Castro et al., 2023). Additionally, MCT can help estimate the long-term fate of established polyploid species

and their competitive interactions with diploid parents (Rey et al., 2017). MCT predicts the coexistence of two cytotypes based on two mechanisms: stabilizing mechanisms, which increase niche difference, and equalizing mechanisms, which reduce the average fitness gap between cytotypes (Adler et al., 2007; Chesson, 2000). In the context of intercytotypic competition, niche differences would represent the average extent to which cytotypes restrict individuals of their own cytotype compared to those of other ploidy levels. Moreover, fitness differences underlie competitive dominance and, in the absence of niche distinctions, determine competitive superiority between pairs of cytotypes.

The competitive dynamics between cytotypes can be influenced by various factors. Niche and fitness differences are not static, as they depend on the abiotic conditions under which cytotypes compete (Germain et al., 2018). Consequently, competitive dynamics can change with shifting climate conditions (Van Dyke et al., 2022). Indeed, climate has been recognized as one of the most influential predictors of the distribution of polyploid species globally (Rice et al., 2019). Thus, understanding the impact of the environment on cytotype coexistence mechanisms is particularly significant under global change scenarios, under which climatic conditions are projected to undergo drastic alterations. This is especially relevant in the Mediterranean region, where an increase in temperature is predicted to be accompanied by severe and more frequent drought episodes over the coming years (Giorgi & Lionello, 2008).

Another factor influencing competitive interactions, alongside the environment, is the divergence of functional traits between cytotypes. Polyploidization often induces significant changes in cell size and adjustments in gene regulatory networks (Ebadi et al., 2023), leading to morphological and physiological alterations with substantial ecological and evolutionary implications (Li et al., 1996; Weiss-Schneeweiss et al., 2013). Genome duplication results in larger stomatal guard cells and lower stomatal density, which would positively impact photosynthetic rates (Maherali et al., 2009; Vyas et al., 2007; Zhang et al., 2020). Moreover, polyploids tend to have a lower osmotic potential and a thicker epidermis (Correia et al., 2023; Li et al., 1996), which generally increases drought resistance, potentially conferring polyploids a competitive advantage over diploids in water-limited scenarios (Aschehoug et al., 2016; Kraft et al., 2015). This variation in

functional traits may influence fitness so that trait divergence is expected to underlie the nature and magnitude of competitive interactions between polyploids and diploids. Previous studies have investigated the relationship between functional traits and competitive interactions (Cadotte, 2017; Kraft et al., 2015; Kunstler et al., 2016; Pérez-Ramos et al., 2019). Indeed, the specific leaf area (SLA), an effective functional trait widely used as a proxy for plant growth strategy (Quétier et al., 2007; Wright et al., 2004), is globally correlated with low competitive effect (Kunstler et al., 2016). However, the contribution of functional traits to competitive dynamics in the context of polyploidy has been understudied (but see Guo et al., 2023). Therefore, it would be valuable to elucidate whether divergence in functional traits or the traits themselves modify competitive interactions between cytotypes under contrasting water availability scenarios.

The autopolyploid *Dianthus broteri* complex (Caryophyllaceae) is an excellent system to unravel the competitive dynamics between polyploids and diploids and the modulating impact of water availability and functional traits. Endemic to the Iberian Peninsula, this polyploid complex comprises four cytotypes (2x, 4x, 6x, and 12x) that form independent monophyletic lineages and are distributed in monocytotypic populations with disjunct geographic ranges (Balao et al., 2009, Balao et al., 2011). Their distinct ecological niches are distributed along an aridity gradient, with the highest ploidy levels (6x and 12x) occupying the most restricted and extreme habitats (López-Jurado et al., 2019). The presence of monocytotypic populations in *D. broteri*, along with a single known secondary 4x-12x contact zone (Balao et al., 2009), indicates an ongoing unstable coexistence between cytotypes. Moreover, cytotypes exhibit incipient divergence in vegetative and reproductive traits (Balao et al., 2011), photochemical responses (López-Jurado et al., 2020), temperature tolerances (López-Jurado et al., 2024) and ecological strategies (López-Jurado et al., 2022). Specifically, 6x and 12x showed the greatest divergence in terms of ecological strategies where 6x showed a nutrient and water conservation strategy (i.e., low SLA) and 12x showed a nutrient and water acquisition strategy (i.e., high SLA). Therefore, examining the way these and other physiological functional traits influence competitive dynamics between cytotypes could provide fundamental insights into the mechanisms underlying these interactions, especially in the context of regional water deficits.

The present study examines the coexistence of distinct cytotypes within common environments, focusing on the impact of water availability and trait divergence. We

hypothesize that water availability would modify the competitive dynamics among cytotypes. Specifically, cytotypes with lower ploidy levels (2x and 4x) would dominate under HWA, while cytotypes with higher ploidy levels (12x and 6x) would dominate under LWA. Consequently, the coexistence of cytotypes will be affected too. Furthermore, both trait divergence and inherent traits will influence the competitive interactions among cytotypes. To test these hypotheses, we (1) describe changes in the competitive ability of cytotypes under two contrasting soil water availability scenarios, (2) predict coexistence between cytotypes by analyzing fitness and niche differences, and (3) elucidate potential linkages between functional traits and competitive interactions, thereby providing insights into their competitive interactions under arid conditions.

Material & Methods

Experimental competition set-up

We sowed 690 seeds from three populations of each *D. broteri* cytotype to ensure the highest possible representativeness. The seeds were germinated in Petri dishes in a chamber model F-1 (Ibercex, Spain), under a 12/12 h photoperiod and 25/17 °C. We implemented a 'minimal target-neighbour' design (Hart et al., 2018) to quantify cytotype competition by pairing two individuals in each combination. Thus, we used 14 competitive combinations (2x-2x, 2x-4x, 2x-6x, 2x-12x, 4x-4x, 4x-6x, 4x-12x, 6x-6x, 6x-12x, 12x-12x with four monospecific stands 2x, 4x, 6x, and 12x) under two contrasting water availability regimens. Under high water availability (HWA), soil moisture was maintained between 40% and 45% of the soil water content (% VWC), while under low water availability (LWA), soil moisture was maintained between 15% and 20% VWC. This setup allowed us to examine the effects of intraploidy and interploidy competition within the *D. broteri* complex, and how these ploidy interactions could vary by the availability of soil water.

We assigned 480 seedlings from each cytotype to the 28 experimental combinations (14 competitive combinations × two water availability treatments) and grown in 650 mL pots filled with a mixture of a commercial organic substrate (Gramoflor GmbH und Co. KG.) and perlite (3:1) and placed in a greenhouse with controlled minimum and maximum temperature of 16-26 °C, 40-60 % relative humidity and unfiltered natural light conditions (approximately 14/10 h day/night and maximum photosynthetic photon flux density

incident on leaves of 1000 μmol m^{-2} s^{-1}). During the experiment, VWC of each pot was measured regularly using an ML3 ThetaProbe soil moisture sensor (Delta-T Devices Ltd) to check soil water levels.

Functional trait measurements

After six months of growth under the previously described conditions, six replicates from each competitive combination survived, totalling 168 competitive units. The total dry biomass (above- and belowground) of each individual (n = 378) was quantified after drying for 48 h in a fan-forced oven at 65°C. Additionally, we measured a set of functional traits from each competition combination (apart from the monospecific stands). We performed instantaneous gas exchange measurements in a randomly selected, fully developed leaf per individual (n = 242) using an IRGA gas exchange meter (LI-6800, LI-COR Inc., Neb., USA). We quantified the net photosynthetic rate (A_N), stomatal conductance (g_s), and intrinsic water use efficiency ($_i$WUE) under the follow leaf cuvette conditions: 400 μmol mol^{-1} [CO_2], 25/28 ºC, 50 ± 5% relative humidity, and a photon flux density of 1000 μmol photon m^{-2} s^{-1} (with 15% blue light to maximize stomatal aperture). Furthermore, we investigated the maximum quantum efficiency of PSII photochemistry (F_v/F_m) using a portable modulated fluorometer (FMS-2, Hansatech) with dark-adapted leaves (n = 263) measured at noon (1600 μmol photon m^{-2} s^{-1}). In addition, specific leaf area (SLA; leaf area per unit leaf dry mass) and leaf dry matter content (LDMC; leaf dry mass per unit leaf fresh mass) were determined (n = 275) following the protocols described by Cornelissen (2003). Leaf area was measured using ImageJ software (US National Institutes of Health, Bethesda, MD, USA) from scanned leaves and samples were oven-dried at 65 °C for 48 hours.

Competitive interactions and the effect of water availability on the MCT

We estimated different competition parameters in the MCT framework (Chesson, 2012) using the *cxr* package ver. 1.0.0 (García-Callejas et al., 2020) in R ver. 4.3.3 (R Core Team, 2024. Our experiment was designed to parameterize a mathematical model describing the population dynamics of interacting species (Ricker, 1954), which was extended here to cytotypes. This model, based in the per-capita population growth rate, incorporated the competitive interactions between cytotypes (α_{ij} and α_{ii} for inter- and intracytotypic competition respectively) and their demographic parameters (Godoy & Levine, 2014; Levine & HilleRisLambers, 2009). Detailed mathematical explanations for

these parameters are provided in Supplementary Methods 1. We used the total dry biomass (over six months) as proxy for per-capita population growth rate due to the perennial nature of the species and the controlled conditions of the greenhouse environment (Chung & Rudgers, 2016; Dostál, 2023; Zhang & van Kleunen, 2019). Notably, vegetative biomass shows a high correlation with reproductive biomass in 12x *D. broteri* ($R^2 = 0.85$, p-value < 0.001, $n = 9$). Based on this demographic model, we estimated the intrinsic growth rates, stabilizing niche differences, average fitness differences, and predicted competitive outcomes. Negative niche differences are indicative of priority effects, which implies that cytotypes are experiencing positive density dependence and suggesting that the first species to arrive in a community gains an advantage. The niche differences between 0 and 1 reflect the degree to which cytotypes limit each other compared to themselves. Furthermore, when there is complete niche overlap, the dominant cytotype is solely determined by its average fitness ratio. The fitness difference was estimated from its two components, the demographic ratio and the competitive response, which offer alternative routes to competitive dominance (Godoy & Levine, 2014). The demographic ratio refers to the proportion of intrinsic growth rates between cytotypes, while the competitive response refers to the degree to which a cytotype is susceptible to intracytotypic and intercytotypic competition. We tested the effect of water availability on niche and fitness differences for each cytotype competitive combination using a two-tailed paired Student's t-test. Furthermore, based on the niche and fitness differences, we investigated the three possible outcomes for the coexistence of cytotypes: stable coexistence when the differences in the niche exceed the differences in fitness, competitive exclusion when the differences in fitness exceed the differences in the niche, or priority effects when the differences in the niche are negative and it is predicted that the first cytotype to arrive in a community gains an advantage (Ke & Letten, 2018). Lastly, from the fitness difference parameter, we also derived the competitive ability (Hart et al., 2018) for each cytotype, which assesses the fitness of each cytotype against all other competitors, assuming null niche differences (see Supplementary Methods 1 for details). A higher competitive ability can arise from the ability to achieve a large biomass in the absence of competition (i.e., higher intrinsic growth rate) and/or a lower sensitivity to competition with other cytotypes (i.e., the geometric mean of α_{ij} and α_{ii}).

Divergence of functional traits in polyploids and its role in competition

To evaluate the divergence in functional traits between cytotypes and the effects of competition (presence/absence) and water availability (HWA/LWA) on these traits, we used generalized linear models (GLMs) with a Gaussian error structure and an identity link function for each dependent variable. Models were fitted to examine the independent effects of each factor and their two-way interactions. Mean trait values for significant factors were compared with Tukey's post hoc tests (α = 0.05) using the *lsmeans* package (Lenth, 2016). Additionally, we explored the complex relationships between traits and competition by expanding the Ricker competition model. We adapted the neighbourhood modelling framework from Kunstler et al. (2016) to our greenhouse experimental setup. In our model, the growth of the focal cytotype (f) is represented as the product of its maximum potential growth (which includes the effects of traits on growth), and the reductions caused by competition from the neighbouring cytotype (c). To quantify this phenomenon, we used the following equation:

$$B_{f,p,w} = Bmax_{f,p,w} \exp\left(-\alpha_{f,c} B_{c,p,w}\right)$$

Here, $B_{f,p,w}$ represents the biomass of the focal individual (f) in competition while $Bmax_{f,p,w}$ is its maximum biomass in the absence of competitors modelled with a normally distributed random effect of ploidy level (εp), in which $\varepsilon p \sim \mathrm{N}(0, \sigma_\gamma)$, and random effect of watering (εw), in which $\varepsilon w \sim \mathrm{N}(0, \sigma_\gamma)$. The competitive parameter, $\alpha_{f,c}$, is the effect of the neighbour cytotype (c) on the growth of the focal cytotype (*f*), which is multiplied by the biomass of the competitor ($B_{c,p,w}$). We modelled the effect of traits on the growth model parameters individually, assessing one trait at a time. The effect of a focal cytotype trait value (t_f) on its maximum growth was represented as $m_0 + m_1 t_f$. Here, m_0 denotes the average maximum growth, and m_1 represents the effect of the focal cytotype trait. The competitive parameter ($\alpha_{f,c}$) was modelled using an equation of the form:

$$\alpha_{f,c} = \alpha_{ointra,f} C + \alpha_{ointer,f}(1 - C) - \alpha_t t_f + \alpha_e t_c + \alpha_d \left|t_c - t_f\right|$$

In which $\alpha_{ointra,f}$ and $\alpha_{ointer,f}$ are (respectively) trait-independent intraspecific and trait-independent interspecific average competition for the focal cytotypes (*f*), each modelled with a normally distributed random cytotypes effect (ε_f) as: $\alpha_{ointra,f} = \alpha_{ointra,f} + \varepsilon f$. The parameter *C* is a binary variable that takes the values 0 when the

combination is intercytotypic and 1 when it is intracytotypic; α_t represents the competition tolerance (or competitive response) of the focal species, indicating the alteration in competition tolerance attributed to the traits (t_f) of the focal individual; α_e represents the competitive effect, denoting the alteration in competition effect caused by the traits (t_c) of the neighbouring individual, while α_d signifies the effect of trait dissimilarity, indicating the change in competition due to the absolute distance between traits $|t_c - t_f|$. Finally, it should be noted that the values of $\alpha_{f,c} > 0$ indicate competition, while $\alpha_{f,c} < 0$ indicates facilitation.

To estimate the standardized coefficients for each model, both the response and explanatory variables were standardized by dividing them by their respective standard deviations before analysis. We conducted two versions of each model. In the first version, the parameters (m_0, m_1, α_0, α_t, α_e, α_d) were estimated as constants across both water availability treatments (HWA and LWA). In the second version, we allowed for different fixed estimates of these parameters for each water availability, enabling us to examine the variation between the two experimental conditions more closely. The trait-based neighbourhood models were fitted by dynamic Hamiltonian Monte-Carlo sampling, using Stan v.2.32.2 (Stan Development Team 2024), with the package *rstan* v.2.32.6 as a front-end. We fitted the survival and growth models jointly, running five chains of 10000 iterations, including 5000 iterations of adaptation. Weakly informative priors were used for all parameters.

Results

Competitive interactions are largely affected by water availability

Our results showed that soil water availability significantly influenced the competitive abilities of cytotypes (Fig. 1 a, b). Under HWA (Fig. 1a), the tetraploid cytotype (4x) exhibited the highest competitiveness, followed by the diploid (2x), hexaploid (6x), and dodecaploid (12x). However, under LWA, this order was reversed. The 6x cytotype

demonstrated the highest dominance, followed by the 12x, 2x, and 4x, respectively (Fig. 1b).

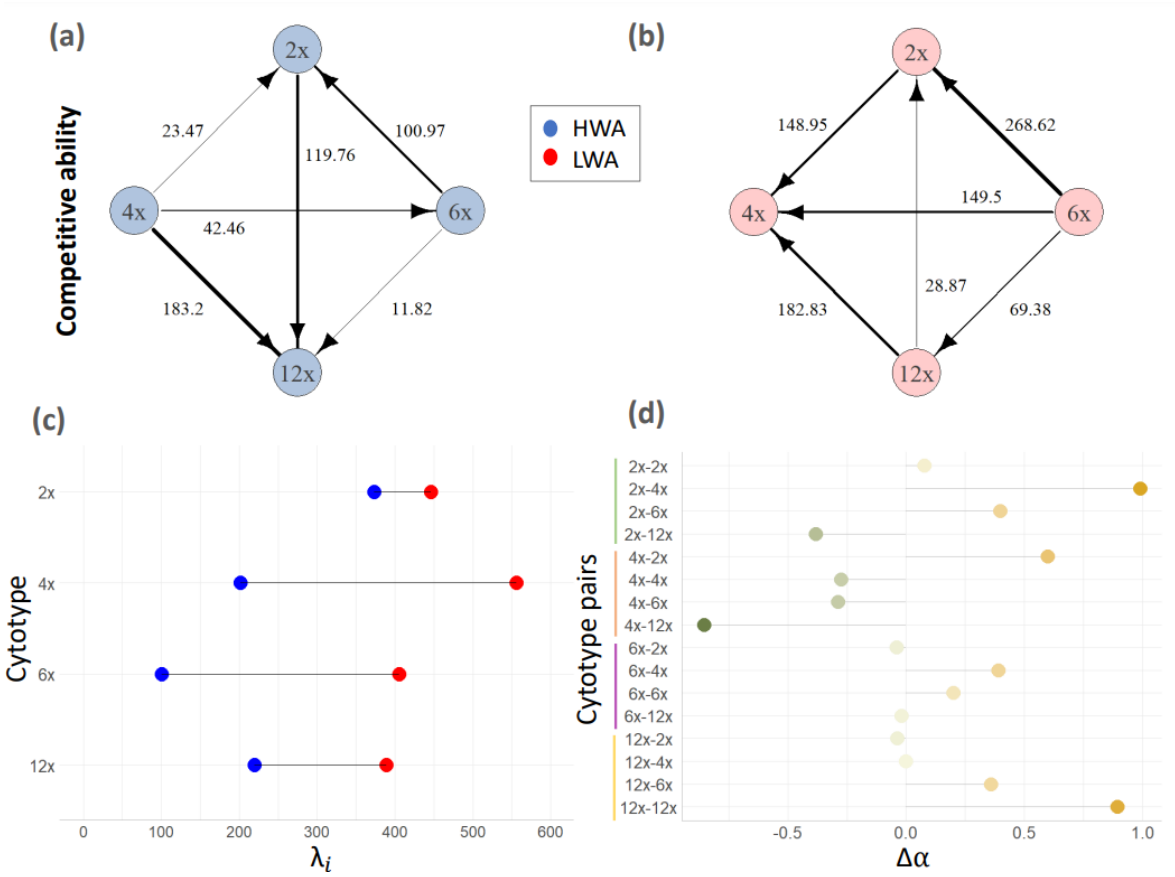

Fig. 1. The competitive ability of *Dianthus broteri* cytotypes (2x, 4x,6x and 12x) is shown under both high **(a)** and low water availability **(b)** conditions. The direction of the arrow indicates the cytotype with the lowest competitive ability in each combination, while the thickness of the arrow denotes the magnitude of the fitness differences. Changes in the direction of the arrows indicate changes in the dominance between climate scenarios. **(c)** Intrinsic growth rate (λ_i) of the different cytotypes in the absence of competition (blue dots indicate HWA conditions, while red dots indicate LWA conditions). **(d)** Difference between interaction coefficients (α_{ii} and α_{ij}) in HWA and LWA conditions ($\Delta\alpha = \alpha_{HWA} - \alpha_{LWA}$). Positive differences (orange dots) suggest higher interaction coefficients under HWA condition compared to LWA condition, while negative differences (green dots) suggest the opposite pattern.

When competitive ability was decomposed into intrinsic growth rate (i.e., biomass in the absence of competition) and sensitivity to competition (i.e., the geometric mean of intra- and interspecific interaction coefficients), we observed a significant increase in intrinsic growth rates across all cytotypes under LWA (paired t-test t = 3.5137, df = 3, p-value <0.05 Fig. 1c). However, the dominant cytotype (with the highest competitive ability) under each water availability condition (4x under HWA and 6x under LWA) did

not exhibit the highest intrinsic growth rate compared to other cytotypes, but rather showed a lower sensitivity to competition (Fig.1d). The LWA markedly altered the intensity of both intra- (α_{ii}) and interspecific (α_{ij}) competition but those differed between cytotype pairs (Fig. 1d). The tetraploids under HWA had overall remarkably low α_{ii} and α_{ij} (except for their interaction with diploids), which explains their high competitive ability. Conversely, the tetraploids exhibited the lowest competitive ability under LWA as a consequence of increased interaction coefficients (specifically, 4x-12x pair). In turn, the hexaploids showed the highest competitive ability under LWA due to their higher tolerance to competition, as evidenced by their notably low interaction coefficients (Fig. 1d). Additionally, the dodecaploid cytotype showed a substantial disparity in its intraspecific interaction coefficient which was notably higher under HWA compared to LWA. Consequently, the 12x cytotype displayed the lowest competitive ability under HWA due to its increased sensitivity to competition. Moreover, for the diploids, its competitive ability was not affected by the water availability treatment, since in both cases it was intermediate.

Similarly, niche difference was significatively higher in HWA than LWA (paired t-test, $t = -3.9509$, $df = 5$, p-value <0.05; Fig. 2), whereas the fitness difference showed the opposite pattern (paired t-test, $t = -3.5499$, $df = 5$, p-value <0.05; Fig. 2). Nevertheless, the feasibility of cytotype coexistence was rarely predicted with only one pair of cytotypes coexisting in each water availability condition (Fig. 2). Under HWA, our model predicted coexistence of the 4x-12x pair mainly due to high niche differences. In the other combinations (4x-2x and 4x-6x), the tetraploids tended to dominate, excluding other cytotypes. In contrast, under LWA conditions, the model predicted the coexistence of 2x and 4x cytotypes (given their high niche difference and low fitness difference). Meanwhile, the hexaploids outcompeted the other cytotypes –including tetraploids– under LWA, leading to their competitive exclusion. Additionally, the 12x-2x pair exhibited strong priority effects due to negative values in the niche difference (Fig. 2).

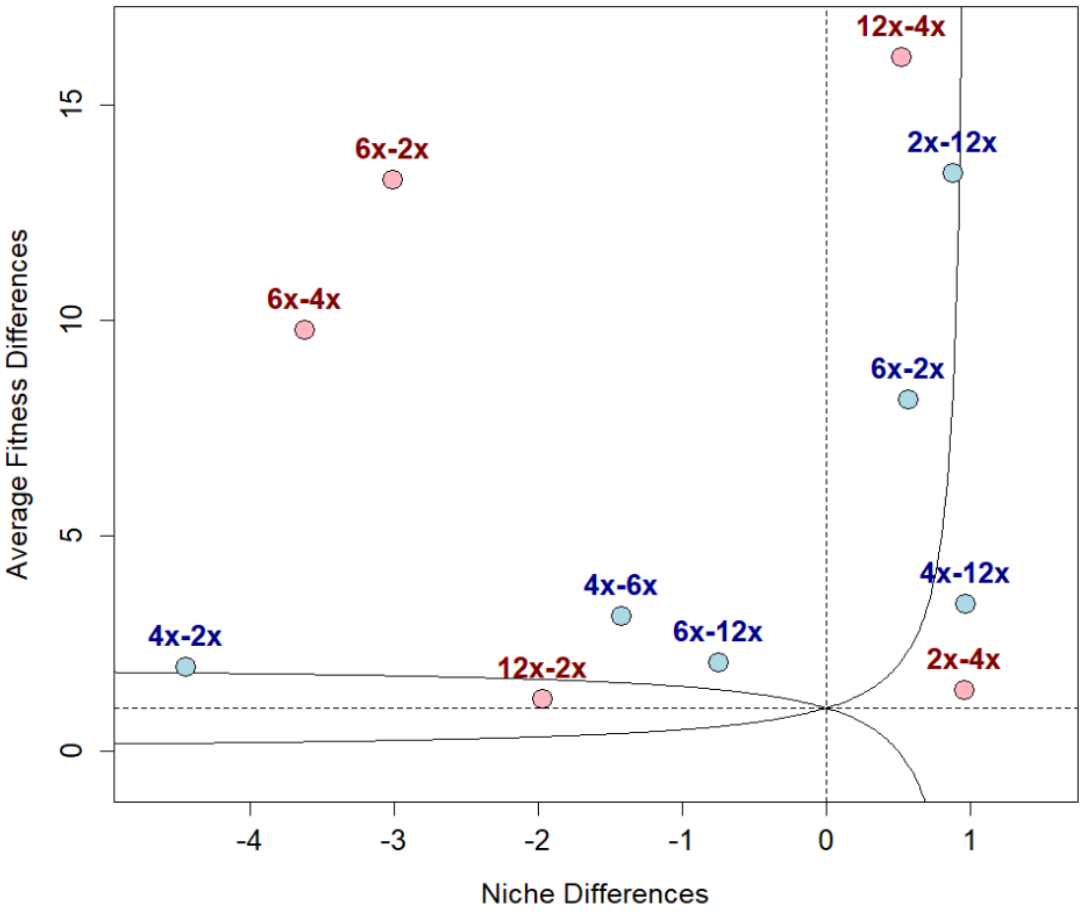

Fig. 2: Relationship between fitness difference and niche difference for each competitive interaction between *Dianthus broteri* cytotypes (2x, 4x, 6x, and 12x) in HWA (blue dots) and LWA conditions (red dots). The two continuous black lines represent the coexistence condition and its symmetry, delineating the space where cytotypes could coexist and where priority effects occur. Outside the coexistence zone, the first cytotype of each combination will be the one that excludes the second of that combination. The 6x-12x pair under LWA is excluded from the graph due to its niche difference of -18.

Functional trait divergence

Our results showed that water availability had a significant impact on all functional traits **(Table 1).** Specifically, for A_N, g_s, LDMC and $_i$WUE the LWA treatment increased their value by 9.13-109.15%. In contrast, for SLA and F_v/F_m it decreased their value by 8.13-67.8%. Furthermore, cytotypes showed substantial variations in g_s, SLA, and LDMC, with significant differences being specific to each cytotype pair. We observed significant differences in g_s between 4x (112.69 ± 10.23 mmol H_2O m^{-2} s^{-1}) and 12x (154.45 ± 10.49 mmol H_2O m^{-2} s^{-1}; Tukey's post hoc test; p-value < 0.05). SLA differed between 2x (11.44 ± 0.453 m^2 Kg^{-1}) and 12x (19.963 ± 2.489 m^2 Kg^{-1}; Tukey's post hoc test; p-value < 0.05).

In addition, LDMC showed differences in 2x (309.59 ± 6.78 g) and 6x (259.19 ± 11.04 g; Tukey's post hoc test; p-value < 0.05) and between 2x (309.59 ± 6.78 g) and 12x (263.60 ± 9.19 g; Tukey's post hoc test; p-value < 0.05). Alternatively, the interaction between ploidy level and water availability was not observed to be significant for any of the traits. Similarly, competition had no significant independent effect on any functional trait, nor did the interaction between competence and water availability treatment (Table 1). However, the interaction between competition and ploidy level led to significant divergence in g_s, where the values decreased when 2x plants were in competition (111.0756 ± 10.80 mmol H20 m^{-2} s^{-1}) compared to when they were grown alone (163.47 ± mmol H20 m^{-2} s^{-1}; Tukey's post hoc test; p-value < 0.05).

	df	A_N	g_s	SLA	LDMC	F_v/F_m	$_i$WUE
Competition	1	0.2635	0.3854	0.0426	0.0978	0.0051	0.0222
Water availability	1	25.1462*	25.375*	14.2780*	4.9718*	13.7343*	9.7650*
Ploidy	3	1.9683	2.8713*	4.1760*	6.41*	1.2733	0.5628
Competition:Water availability	1	0.0153	0.119	0.2119	0.0791	0.6581	0.8269
Water availability:Ploidy	3	2.5397	2.5918	2.9411	0.4390	0.1053	0.4435
Competition:Ploidy	3	0.6563	3.221*	4.9168	2.364	0.5432	1.1277

Table 1: Results of Generalized Linear Model (GLM) analyses examining the association between functional traits and ploidy levels (2, 4x, 6x and 12x), competition (alone or with competitor), water availability (high and low) along with their pairwise interactions. Degrees of freedom (df) and F-value are shown, with significant results (p-value < 0.05) indicated by an asterisk.

Functional traits promote tolerance and competitive effects

Our model demonstrated that functional traits shape competitive effects in the *D. broteri* complex, with soil water availability significantly influencing these relationships. These trait effects are driven by four main mechanisms. Firstly, LMDC and SLA traits were directly associated with higher and lower growth rates, respectively, in the absence of competition (m_1, Fig. 3). These global effects were influenced by water availability, being pronounced for SLA under HWA and for LMDC under LWA. Furthermore, lower values of A_N, F_v/F_m and $_i$WUE were associated with increased tolerance to competition from neighbouring cytotypes (i.e., 'competitive response'; $\alpha_t t_f < 0$, Fig. 3). The competitive response of these traits persisted under LWA but not under HWA. Additionally, a stronger competitive effect ($\alpha_e t_c$) would enable cytotypes with higher trait values to suppress the

growth of neighbouring cytotypes more effectively, SLA under LWA conditions exerted a significant positive competitive effect ($\alpha_e t_c > 0$, Fig. 3). Lastly, our analysis revealed a global increased trait dissimilarity effect (α_d $|t_c\text{-}t_f|$) for A_N and LDMC, which led to decreased competition and reduced suppression of focal cytotype growth. This effect was maintained under both water availability conditions for LDMC and under LWA for A_N ($\alpha_{d\ |tc\text{-}tf\ |} > 0$, Fig. 3).

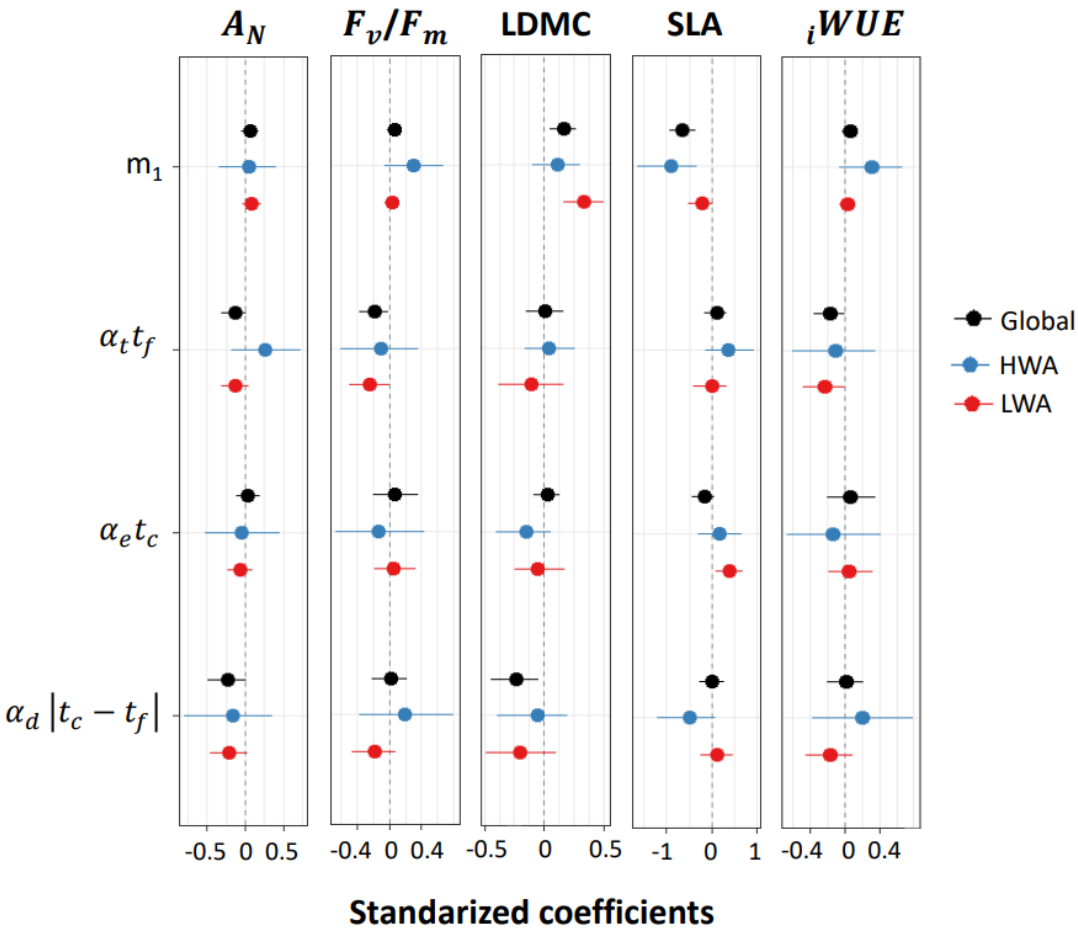

Fig. 3: Standardised regression coefficients for growth models, adjusted separately for net photosynthetic rate, A_N, maximum quantum efficiency of PSII photochemistry F_v/F_m, leaf dry matter content, LDMC, specific leaf area, SLA and intrinsic water use efficiency $_i$WUE. Stomatal conductance (g_s) is not shown as it is not significant for any competition parameter. Dots represent average estimates, while lines indicate 95% confidence intervals. Dark dots and lines depict joint control and drought estimates, blue represents HWA conditions, and red represents LWA. Parameter estimates include: the effect of the focal individual trait (t_f) value on maximum growth (m_1), the effect of the focal cytotype trait value (t_f) on its tolerance to competition (α_{t_tf}) (positive values indicate higher trait values result in greater tolerance to competition), the effect of trait values in the individual competitor (t_c) on its competitive effect (α_{e_tc}) (positive values indicate higher trait values lead to greater reduction in focal cytotype growth) and the effect of trait

dissimilarity between the focal cytotype and its competitors ($\alpha_{d|t_c-t_f|}$) (negative values indicate greater dissimilarity leads to less reduction in focal cytotype growth). Significant estimates are those that do not cross the zero line.

Discussion

Competitive interactions are largely affected by soil water availability

While numerous studies have reported differences in competitive ability between polyploids and diploids, usually based on indirect evidence (Castro et al., 2023; Čertner et al., 2019; Grewell et al., 2016), other studies have found no significant evidence to support these differences (Hülber et al., 2011; Münzbergová, 2007). Our study investigates the fine-scale competitive dynamics between polyploids and diploids in the *Dianthus broteri* complex, focusing on the implications for the long-term persistence of polyploids and their response to variations in soil water availability. We employed recent advancements in ecological coexistence theory and parameterized models, demonstrating that both polyploidy and water availability significantly affected most competition parameters. In line with polyploids generally experiencing delayed ontogenesis and exhibiting a reduced rate of biomass accumulation (Corneillie et al., 2019; Deng et al., 2012), we found here that the intrinsic growth in higher cytotypes (6x and 12x) was consistently lower than in lower ploidy level cytotypes (2x and 4x; Fig 1c). This phenomenon, referred to as "high-ploidy syndrome" (Tsukaya, 2008), has been associated with an elevated demand for resources in lineages after genome duplication (Anneberg & Segraves, 2023; Guignard et al., 2017) However, there are studies that suggest the opposite, indicating that polyploids exhibit a faster growth rate and larger organs size (Šmarda et al., 2023; Sugiyama, 2005).

Additionally, consistent with the known effect of drought on interspecies competition (Adler et al., 2012; López et al., 2013), our results suggest that water availability induces changes in the network of competitive interactions within the *D. broteri* complex (Fig. 1a, b). Under HWA, presumably the most benign condition, the tetraploids exhibited the highest competitive ability. This finding aligns with the observation that 4x is the most geographically widespread cytotype (Balao et al., 2009; López-Jurado et al., 2019). Conversely, the highest ploidy levels (12x and 6x) demonstrated the lowest competitive ability, consistent with their restricted distribution

to the most arid areas. Congruently, under LWA, the competitive hierarchy was reversed, with 6x and 12x emerging as superior competitors. This finding is supported by previous studies on *D. broteri* that underscored the adaptation of its higher ploidy cytotypes to stressful conditions (López-Jurado et al., 2020, 2024) and the importance of certain ecophysiological traits previously described (i.e., F_v/F_m, relative water content or leaf water potential) for drought tolerance (López-Jurado et al., 2016). While these results are consistent with previous studies indicating that water deficit can enhance the competitive ability of polyploids over diploids (Guo et al., 2023; Rey et al., 2017), no differences in competitive abilities between diploids and tetraploids were observed under reduced soil moisture in *Chamerion angustifolium* (Thompson et al., 2015).

Competitive exclusion is mediated by soil water availability

As indicated in the competitive outcomes analysis, our competition model predicted a general pattern of competitive exclusion among *D. broteri* cytotypes under secondary contact. This is congruent with the allopatric distribution of the cytotypes (Balao et al., 2009) and their divergence in ecological niches (López-Jurado et al., 2019), suggesting that intercytotypic competition is shaping their current distributions. Similarly, the Mediterranean *Brachypodium distachyon* and other polyploid complexes have exhibited this intercytotype exclusion pattern (Buggs & Pannell, 2007; Ćertner et al., 2017; Rey et al., 2017), while stable coexistence, at least in the short term, has been found in other mixed-ploidy species (Kao & Parker, 2010; Keeler, 2004). In *D. broteri*, soil water availability affected competitive interactions between cytotypes and ultimately their predicted coexistence outcomes through modifications in niche and fitness differences, as observed in other studies (HilleRisLambers et al., 2012; Matías et al., 2018; Wainwright et al., 2019). Specifically, under LWA, fitness differences increased, and niche differences decreased, collectively reducing the likelihood of coexistence. Notably, our model predicted the stable coexistence of 4x and 12x cytotypes in HWA, which showed the sole secondary contact zone of the complex found in nature (Balao et al., 2009). This stable coexistence would be possible given the poor competitive ability displayed by the rare 12x, due to its strong self-limitation (Fig. 1d; Chesson, 2000; MacArthur, 1970; Mcpeek, 2012). However, under LWA, the 2x-4x coexistence was also predicted and the possibility of current or new stable secondary contact zones should not be dismissed based on their close distributions and overlapping ecological niches (Balao et al., 2009).

Additionally, the role of chance in the long-term of survival of polyploids has been overlooked (Mortier et al 2024). Founders that establish successfully can secure available resources and prevent the establishment of later-arriving cytotypes with similar niche preferences, regardless of their comparative fitness (Kardol et al., 2013). This priority effect ensures the continued dominance of the initially established cytotype as predicted for the 2x-12x interaction. Nevertheless, these priority effects were again modulated by water availability, hindering the long-term fate predictions. As water availability increases, the risk of local extinction for higher *D. broteri* polyploids (6x-12x) also rises. Conversely, under the predicted future aridity scenario for the Iberian Peninsula, these higher polyploids (6x and 12x) are expected to dominate the competition and expand, outcompeting lower ploidy cytotypes and stabilizing in the long term (Ræbild et al., 2024). This scenario is consistent with the non-random establishment and long-term survival of many polyploid lineages, which have coincided with major periods of global climate change and high stress (Cai et al., 2019; Van De Peer et al., 2017; Van de Peer et al., 2021). In fact, temperature and rainfall are among the most influential predictors of polyploid distribution, with polyploids exhibiting lower frequencies from the poles towards the equator and in regions with high precipitation seasonality (Rice et al., 2019).This apparent paradox is resolved by the role of water stress as an evolutionary driver, with polyploids being better adapted than their diploid progenitors (Del Pozo & Ramirez-Parra, 2014; Yang et al., 2021; Zhang et al., 2015), due to changes in phenotypic traits associated with transpiration and photosynthesis (Tossi et al., 2022). These adaptations explain the notable association between dry habitats and polyploidy across diverse lineages of land plants (López-Jurado et al., 2019; Rey et al., 2017; Te Beest et al., 2012).

Functional traits modulate competitive effects and response

Phenotypic changes resulting from polyploidization, particularly those related to physiological plasticity, can confer fitness advantages and modify competitive effects under water shortage (Guo et al., 2023). Our analysis revealed significant inter-cytotype differences in g_s, SLA, and LDMC, which is in accordance with previous studies on the *D. broteri* complex (López-Jurado et al., 2022, 2024). Notably, the 4x cytotype exhibited a significantly lower g_s compared to the 12x, as also observed under heat waves, suggesting divergent stress response strategies (López-Jurado et al., 2022, 2024). Furthermore, previous studies suggest that the 12x cytotype appears to employ a

physiological avoidance mechanism, due to sufficiently high levels of g_s to maintain constant relative water content and leaf water potential (López-Jurado et al., 2016), which could explain its competitive dominance over 4x under abiotic stress (Fig. 1ab).

Similarly, our trait-based neighbourhood analyses confirmed the direct and indirect roles of the functional traits mediating competitive interactions (Godoy & Levine, 2014; Kraft et al., 2015). We observed a direct relationship between LDMC and growth rate in the absence of competition under LWA. Higher LDMC values imply thicker and stiffer cell walls, allowing the maintenance of turgor at lower leaf water potentials (Cheung et al., 2011; Zimmermann, 1978). This anatomical feature would then minimize cell damage during drought (Bowman & Roberts, 1985), consequently mitigating some of its adverse effects (Blumenthal et al., 2020). Strikingly, despite most studies suggest a strong positive relationship between SLA and the relative growth rate in woody species(Cornelissen et al., 1998; Lambers & Poorter, 1992; Poorter et al., 1990), our results revealed an opposite negative relationship in both HWA and LWA scenarios. This discrepancy may be attributed to the species-specific growth habit variations, as negative relationship between SLA and plant size and height has been observed in herbaceous species (Kleyer et al., 2019). Focusing on our case, we observed that higher-level polyploids (12x and 6x) exhibited a lower intrinsic growth rate compared to lower-level polyploids (2x and 4x; Fig 1d). Additionally, SLA showed significant divergence between cytotypes (Table 1), with higher-level polyploids possessing higher SLA values. The fact that higher ploidy levels have a high SLA value may be a reflection of greater surface investment, which will allow it to release excess temperature through evapotranspiration in more arid habitats (El Nadi, 1974). Therefore, we suggest that polyploidy may be altering patterns of phenotypic covariation (Baker et al., 2024; Balao et al., 2011) by changing the genetic and regulatory bases underlying tr ait expression, leading to new relationships between traits that were previously predictably correlated.

Additionally, functional traits exhibited associations with enhanced competitive effects or improved tolerance to competition, being both predominantly contingent upon water availability. Regarding competitive effects, a modest positive correlation was observed with SLA under LWA, indicating that higher SLA in competitors corresponds to reduced biomass in focal individuals. Species with elevated SLA generally exhibit higher growth rates and shorter lifespans, characteristic of a resource-acquisition strategy on the leaf economics spectrum (Poorter et al., 2009; Wright et al., 2004). This

"acquisitive" strategy, as manifested by competitors, would restrict resource availability for the growth of the focal individual. Furthermore, higher F_v/F_m and A_N, related to the efficiency and capacity of photosynthesis, were correlated with lower tolerance to competition ($\alpha_t t_f$; Fig. 4). This negative relationship suggests a trade-off between the ability of cytotypes to tolerate competition and their performance in the absence of resource competition. Although several studies have documented this trade-off, the underlying mechanisms remain to be elucidated (Campbell & Holdo, 2017; Cope et al., 2021). Similarly, $_i$WUE also exhibited a negative relationship with tolerance to competition. Plants with high iWUE are typically associated with a resource-conservative strategy, where they prioritize longevity over rapid growth (Bacelar et al., 2012). In contrast, in highly competitive environments where water is limited, plants that adopt an acquisitive strategy, characterized by lower iWUE, often have an advantage because they maximize rapid resource capture (Wright et al., 2004). As a result, plants following a conservative strategy (high iWUE) may exhibit lower tolerance to competition because they are less able to respond dynamically to resource availability in competitive environments.

Lastly, trait dissimilarity had little effect on competition between *D. broteri* cytotypes. Dissimilarity in net photosynthetic rate (A_N α_d $|t_c\text{-}t_f|$) led to decreased competition supporting the "competition-trait similarity" hypothesis, which predicts that the intensity of competition between individuals or species increases with the similarity of their ecological and physiological traits (Cadotte et al., 2013; Falster & Westoby, 2003; Wong et al., 2021). Greater differences in functional traits between species would indicate greater niche differentiation (D'Andrea & Ostling, 2016), allowing species to minimize competition and partition the local resource pool despite overlapping in resource use and ecological preferences (Macarthur & Levins, 1967).

Concluding remarks

Our findings underscore that the long-term fate of polyploids is highly dependent on environmental variation, which modulates the strength of competitive interactions and coexistence. Water availability in the soil influences ecological settings such as niche and fitness differences, determining competitive outcomes between cytotypes in *D. broteri*. We predicted pairwise-cytotype competitive exclusion, leading to the observed single-ploidy populations and the displacement of higher cytotypes to arid areas. Dominance of

higher ploidies under water-deficit conditions is predicted, supporting the polyploid advantage in these stressful environments. Additionally, we elucidate links between particular functional traits related to physiological performance (i.e., A_N, F_v/F_m, $_i$WUE and SLA) and tolerance and competitive effect parameters underlying competitive outcomes. Our findings offer valuable insights into how environmental conditions, ploidy levels and their associated functional traits interact to shape ecological relationships and cytotypes distributions, as well as enhance our understanding and prediction of long-term establishment and persistence of polyploids. Provided that these results can be extended to other polyploid complexes with similar growth habits in the Mediterranean region, our study provides important new mechanistic insights into their competitive dynamics.

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Competing interests

None declared.

Author contributions

FB conceived the study and designed the experiment. ARP and FB performed the statistical analyses and modelling. ARP, FB, JLJ and EMN interpreted the results. ARP and FB wrote the first draft of the manuscript. All authors critically contributed substantial revisions to the manuscript and approved the final version.

Data availability

The data that support the findings of this study are openly available in Dryad at doi:

The datasets and code for the method developed here are freely available on GitHub

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