Niche divergence and limits to expansion in the high polyploid *Dianthus broteri* complex

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Summary

- Niche evolution in plant polyploids remains controversial and evidence for alternative patterns has been reported. Using the autopolyploid *Dianthus broteri* complex (2x, 4x, 6x and 12x) as a model, we aimed to integrate three scenarios, competitive exclusion, recurrent origins of cytotypes and niche filling, into a single framework of polyploid niche evolution. We hypothesized that high polyploids would tend to evolve towards extreme niches when low ploidy cytotypes have nearly filled the niche space.
- We used several ecoinformatics and phylogenetic comparative analyses to quantify differences in the ecological niche of each cytotype and to evaluate alternative models of niche evolution.
- Each cytotype in this complex occupied a distinct ecological niche. The distributions were mainly constrained by soil characteristics, temperature and drought stress imposed by the Mediterranean climate. Tetraploids had the highest niche breadth and overlap due to their multiple origins, while the higher ploidy cytotypes were found in different, restricted, non-overlapping niches. Niche evolution analyses suggested a scenario with one niche optimum for each ploidy, including the two independent tetraploid lineages.
- Our results suggest that the fate of nascent polyploids could not be predicted without accounting for phylogenetic relatedness, recurrent origins or the niche occupied by ancestors.

Key words

Mediterranean climate, multiple origins, niche filling, phylogenetic niche conservatism, polyploidy

Introduction

Polyploidization (i.e. the process of acquiring more than two complete sets of chromosomes) is one of the major driving forces in plant evolution (Wendel, 2000; Otto, 2007; Soltis *et al.*, 2015). Neopolyploid establishment may be constrained by a frequency-dependent mating disadvantage compared to their diploid parents, called 'minority cytotype exclusion' (MCE; Levin, 1975). For this reason, polyploids need to outcompete their parents or ecologically differentiate from them ('niche escape'). The genome duplication may drive phenotypic changes (Balao *et al.*, 2011a; Laport *et al.*, 2016; Rey *et al.*, 2017), which can lead to shifts in environmental tolerances and so promote increased competitive abilities or subsequent habitat displacement (i.e. niche divergence with expansion or contraction; Manzaneda *et al.*, 2012; Thompson *et al.*, 2014; Visger *et al.*, 2016).

Despite several decades of studies and recent attempts to unify theoretical frameworks (Parisod & Broennimann, 2016), niche evolution in polyploids remains controversial and evidence for several alternative patterns has been found (Glennon *et al.*, 2014; Visser & Molofsky, 2015; Marchant *et al.*, 2016). Niche expansion in neopolyploids may be helped by the lack of selective constraints on duplicate genes and other novel genetic changes that allow the evolution of new functions (Doyle *et al.*, 2008; Leitch & Leitch, 2008; Wendel, 2015). However, the opposite pattern of niche evolution, niche contraction, has also been suggested (Theodoridis *et al.*, 2013; Kirchheimer *et al.*, 2016). On this latter scenario, polyploids would move to narrower and marginal niches with specific ecological conditions, leading to habitat specialization and promoting new habitat colonization (especially at the extreme edges of environmental gradients; Buggs & Pannell, 2007; Boulangeat *et al.*, 2012). Finally, the absence of ecological niche differentiation between cytotypes could be explained by Phylogenetic Niche Conservatism (PNC), the tendency for lineages to preserve ancestral niche-related traits (Crisp & Cook, 2012), or short-range dispersal (Duchoslav *et al.*, 2010) or small differences in relative competitive abilities (Bulleri *et al.*, 2016). One would expect PNC to be more common in autopolyploids (polyploids formed by within-species genome duplication) because of their higher genetic relatedness to their progenitors (Burns & Strauss, 2011; Glennon *et al.*, 2014), although this theory has scarcely been tested (but see Arrigo *et al.*, 2016).

An explanation for the divergence patterns could be that the niche evolution in a polyploid is ultimately limited by niche filling (Tanentzap *et al.*, 2015), which may act at lineage level (based on its origin) more than merely at cytotype level. The first formed polyploids would fill the unoccupied niche space to avoid competition with their ancestor. In this initial step, nascent polyploids would have a high probability of niche expansion. This fact prevents subsequent higher cytotypes from access to such niches, so newcomers must diverge to find available niches for themselves. Eventually, niche expansion would be less likely since higher ploidies encounter limits to their ecological tolerances (Fig. 1; Araújo *et al.*, 2013). Additionally, multiple origins of polyploids (i.e. recurrent formation) are the rule (Soltis & Soltis, 1999) and would cause an increased genetic, biochemical and physiological diversity, conferring the ability to colonize new environments or achieve a broader geographic range (Treier *et al.*, 2009; McIntyre, 2012; Karunarathne *et al.*, 2018). In this case, local adaptation within cytotype would also play an important role in its ecological differentiation (Maherali *et al.*, 2009; Ramsey, 2011; McIntyre & Strauss, 2017). Therefore, patterns of niche evolution in polyploid complexes are likely to be dynamic and non-exclusive.

A study of a large autopolyploid series, that avoids the effects of hybridization (typical of allopolyploids; Spoelhof *et al.*, 2017), may provide insights into the different processes driving polyploid niche evolution. Such insights may be enhanced if we consider information on the phylogenetic relatedness among populations (i.e. the phylogenetic signal in environmental traits). *Dianthus broteri* (Caryophyllaceae) is an excellent system to investigate these underlying causes of niche evolution. This Iberian endemic complex presents the most extensive autopolyploid series for the genus, with 2x, 4x, 6x and 12x cytotypes that very rarely coexist at the same location. In fact, only one mixed-ploidy population with diploid individuals and a few triploids has been described in southwestern Portugal (Balao *et al.*, 2009). While 6x and 12x cytotypes have independently evolved by single events, the tetraploids have been recurrently originated by two polyploidization events (Balao *et al.*, 2010). Furthermore, the four cytotypes present differentiated geographic distributions (Balao *et al.*, 2009). The diploids occur in two disjunct areas (south of Portugal and the mountainous southeast of Spain) whilst the 4x cytotype inhabits the broadest geographical range, with a southern lineage ($4x_s$) distributed from the southwest of Portugal to the south of Spain, and an eastern lineage ($4x_e$) that occurs in the east of Spain. In contrast, the higher ploidies (6x and 12x

cytotypes) inhabit restricted areas with alleged harsh Mediterranean climate conditions (extreme drought and temperatures) in the southeast and southwest of Spain, respectively. Phenotypic changes in floral and vegetative organs (Balao *et al.*, 2011a) may permit shifts in ecological tolerances and therefore in the ecological niche. Supporting this hypothesis, the dodecaploid cytotype (known as *D. inoxianus*), which is an endangered taxon with a highly specialized pollination (Balao *et al.*, 2007, 2011b; Herrera & Balao, 2015), shows an enhanced tolerance to extreme drought events (López-Jurado *et al.*, 2016).

In the present study, we used several ecoinformatics and phylogenetic comparative analyses (Methods S1) to shed light on the niche evolution underlying the rapid radiation by autopolyploidy in the extant cytotypes of *Dianthus broteri* complex (Balao *et al.*, 2009, 2010). We hypothesize that the higher cytotypes have adapted to more specialized environments whereas the lower cytotypes are distributed encompassing a wider environmental range. Additionally, the multiple origins of tetraploids would have contributed to the development of a broad niche (Fig. 1). In alternative scenarios, the high polyploids would just retain the niche of lower cytotypes, reflecting PNC, and the lineages recurrently originated would share the ecological niche conditions. Thus, we address the following specific questions: Is the current cytotype distribution a consequence of PNC or niche divergence? Are the higher polyploids occupying extreme niches because the lower ploidies have nearly filled the niche space? And have the multiple origins of the tetraploids influenced their current ecological niche locations?

Materials and Methods

Occurrence data and cytotype distribution

Locality information was collected from Global Biodiversity Information Facility (GBIF) using the package 'rgbif' (Chamberlain *et al.*, 2017) in R software vers. 3.4.2 (R Core Team, 2017). We restricted the dataset to accurately georeferenced locations with known herbarium vouchers. We added to this information a few additional localities from our personal observations and 29 populations whose ploidy was confirmed by flow cytometry (Supporting Information Table S1). Duplicate occurrences were removed, and locally dense sampling was reduced by thinning the records to one per 1 km^2 grid cell size resulting in 150 localities in total. We assigned the ploidy for all GBIF records based on the clearly delimited distribution areas of the different cytotypes, which occurred as monocytotypic populations (Balao *et al.*, 2009), and the morphological differences of the vouchers using an ad-hoc single-access key based on Balao *et al.* (2011a). For robustness,

we also replicated all the analyses using a reduced dataset which only consisted of the 29 cytotyped populations (25 populations from Balao *et al.*, 2009 and four new ones).

Environmental data

To obtain a detailed description of the environmental niche characteristics for the locations of the *D. broteri* cytotypes, we used a large up-to-date and high-resolution set of predictor layers from four different databases. We selected variables with a likely relevance to the ecological and physiological conditions and constraints that determine the niches of terrestrial plant species: (1) 19 bioclimatic variables from CHELSA database (Karger *et al.*, 2017) at ~1 km^2 resolution, (2) a selection of 11 climatic and 2 topographic continuous variables from ENVIREM database (Title & Bemmels, 2018) at ~1 km^2 resolution, (3) 7 relevant edaphic variables from SoilGrids database (Hengl *et al.*, 2017) at a resolution of 250 m^2 and (4) the altitude information from WorldClim (Fick & Hijmans, 2017). We removed highly correlated environmental variables in the datasets using the variance inflation factor (VIF) with a threshold of 10 (indicating collinearity). We finally obtained for the complete dataset an equitable set of covariates from the four sources used (6 bioclimatic, 5 environmental plus the 2 topographic and 6 edaphic variables; see Fig. 2a). In the reduced dataset, the selected environmental variables were similar with just two different variables (Table S2; Fig. S1).

Ecological Niche Modelling

We evaluated the potential geographic distribution for *Dianthus broteri* as a single species, for its four cytotypes separately, and we also considered the two independent tetraploid lineages using the MaxEnt (Phillips *et al.*, 2006) algorithm in the 'zoon' R package vers. 0.6 (Golding *et al.*, 2018). We extracted 10,000 random background points within the study area (i.e. Iberian Peninsula) for each model. We used the same general background area for all occurrence inputs to minimize their Relative Occurrence Areas (ROAs; Jiménez-Valverde et al., 2008) and to make model comparisons more reliable and easier to evaluate due to the expected subtle differences between the ROAs of the cytotypes (Lobo *et al.*, 2010). Model accuracy was assessed through the commonly employed k-fold cross-validation method (k = 10). For models with fewer than 25 occurrence records, we used the jackknife validation approach (Pearson *et al.*, 2007). For each model, the mean area under the receiver operating characteristic curve (AUC) was calculated with the 'SDMTools' R package (VanDerWal *et al.*, 2014) and tested against

a null distribution (from 99 replicates) to detect significant deviation from random expectation (Raes & ter Steege, 2007).

Niche comparisons: breadth, overlap, equivalency and similarity tests

To compare the environmental niche of the different ploidies (including the 4x lineages), we calculated the kernel-smoothing densities of each occurrence data along environmental axes from a Principal Component Analysis (PCA-env; Broennimann *et al.*, 2012). For the background, we extracted the environmental data from 10,000 spatially thinned random localities within a buffer of 150 km surrounding presence points. We estimated Levins' measure of niche breadth (Levins, 1968) for each cytotype using the 'ENMTools' package (Warren *et al.*, 2010) in R. Furthermore, we estimated the ecological niche overlap between them and performed the niche equivalency test using the Schoener's *D* statistic (Schoener, 1968) in the 'ecospat' R package (Di Cola *et al.*, 2017). Niche equivalency tests compared the observed overlap of *D* values to a null distribution using 100 replicates and an environmental grid resolution of 500 × 500 pixels. In order to refine the niche comparisons, we also performed niche similarity tests to every pair of non-equivalent niches in both directions (Broennimann *et al.*, 2012). We compared the observed overlap value between two occurrence groups to a null distribution of 100 overlap values of one of them and a randomly simulated niche in the available environmental range of the other niche. Finally, as a validation approach, we ran all niche comparison tests using the reduced dataset.

Environmental and phylogenetic niche conservatism

We additionally investigated patterns of niche conservatism or divergence between cytotypes (encompassing again the tetraploid lineages) using a niche divergence test (McCormack *et al.*, 2010) based on the differences in PCA scores between them compared to the differences in scores for distinct 'background regions' for each cytotype. As all occurrences had the same background region, we simply applied pairwise Student's *t* tests to each score combination in the two main PCA axes. A significant value ($P < 0.05$) supported niche divergence, and the alternative indicated niche conservatism.

In addition, to investigate the effect of phylogeny on niche conservatism (i.e. PNC), we tested for phylogenetic signal on the PCA environmental axes as prerequisite for PNC using the Blomberg's K (Blomberg *et al.*, 2003) in the 'phytools' R package vers. 0.6-20 (Revell, 2012). The phylogenetic relatedness among 25 populations of the

reduced dataset was estimated from a bootstrapped phylogram based on previous amplified fragment length polymorphism (AFLP) data (see Balao *et al.*, 2010, 2011a). Polytomies were resolved in random order using the *multi2di* function from the 'ape' package in R (Paradis *et al.*, 2004). In case of significant phylogenetic signal, we compared the relative fit of different evolutionary models for each PCA axis individually (i.e. univariate models) and together (a multivariate model; Beaulieu *et al.*, 2012). In a similar approach to the one used in Balao *et al.* (2011a), we compared a Brownian motion model of gradual drift (BM) against different Ornstein–Uhlenbeck models (OU) representing stasis or stabilizing selection (Butler & King, 2004). We specifically fitted an OU model with a single optima (OU1) and two multi-optima OU models: the first one with an optimum per ploidy (i.e. four optima in all; OU4), and the other with two optima for the tetraploids (two recurrent origins) and one optimum for the remaining cytotypes (i.e. five optima in all; OU5). Computations were performed with the 'mvMORPH' R package vers. 1.0.9 (Clavel *et al.*, 2015). The models were compared by the weights of their Akaike information criterion values corrected by sample size (AICc). A better fit of an OU model was interpreted as a stronger evidence for PNC compared with the BM model (Losos, 2008; Kozak & Wiens, 2010).

Results

Distribution along environmental gradients

The environmental variation within and among ploidies in *D. broteri* was mainly represented in the first two PCA axes (Fig. 2b-d), explaining respectively 28.2% and 21.3% of the total variation in the environmental space. These PCA axes summarized two environmental gradients that constrained the cytotype distributions. The PC1 axis was related to seasonal environmental variables characteristic of the Mediterranean climate and to soil bulk density (*BLDFIE*) and available water capacity (*AWC*). The 12x cytotype occupied an extreme position in this axis (PC1; Fig. 2c), corresponding to localities with harsh dry summers characterized by low precipitation of warmest quarter (*BIO18*) and *AWC* in soils, and a high topographic wetness index (*topoWet*) and potential evapotranspiration (*PET*) in driest and warmest quarters. Furthermore, this cytotype was also associated to warm summers (high maximum temperature of warmest month, *BIO5*, and mean temperature of driest quarter, *BIO9*) and located in flat terrains (low terrain roughness index, *tri*) with high *BLDFIE* (predominantly sandy soils). The PC2 axis mirrored a general aridity gradient (in terms of annual environmental means and with

severe conditions of temperature and rainfall affecting the wettest period of the year). In this case, the axis mainly constrained the distribution of the 6x cytotype (Fig. 2d), showing the lowest values of annual precipitation (*BIO12*) and the metric of relative wetness and aridity (*climaticMoistureIndex*) and the highest ones of *topoWet* and *PET* in the wettest quarter (dry climate conditions). The warm climate conditions predominant in the niche of this cytotype were characterized by low isothermality (*BIO3*) and high *BIO5* and mean temperature of wettest quarter (*BIO8*). Moreover, the hexaploids inhabit poor (low organic carbon content, *ORCDRC*), not sandy (low sand content, *SNDPPT*) and alkaline (high pH, *PHIHOX*) soils. In this environmental space, 2x and $4x_{pool}$ ($4x_e$ + $4x_s$) cytotypes were not clearly differentiated from each other. Whereas pooled tetraploid lineages encompass the complete range of the environmental conditions, diploids are located in rich, sandy, acid soils with more benign characteristics (the lowest position in the PC2 axis). However, the two tetraploid lineages ($4x_s$ and $4x_e$) were environmentally distant from each other. The conditions of the southern tetraploids ($4x_s$) resembled those of 2x and 12x cytotypes (suffering harsher summers with low values in both axes; Fig. 2b-d), whereas the eastern tetraploids ($4x_e$) occur in the right extreme of the PC1 axis and near the 6x populations in the PC2 axis (Fig. 2b-d; milder summers, with non-sandy and watered soils, but several arid conditions throughout the year). These results were robust as similar ones were found using the reduced dataset (Fig. S1). In this last analysis, the two axes described the same environmental gradients but they maximized the environmental distance between cytotypes/lineages.

Environmental suitability and hotspots

The niche models for *D. broteri* complex and for each cytotype revealed considerable niche suitability in the southern and south-eastern Iberian Peninsula (Fig. 3). All cytotypes showed a restricted niche with suitability hotspots solely surrounding actual presence locations (i.e. potential niche is really close to realized niche). As expected, the tetraploid niche merged additively the southern and eastern tetraploid niches (Fig. S2). All these models, even for the $4x_e$ and the 6x with only 20 and 21 occurrences, respectively, obtained high AUC values (> 0.95) which were significantly higher than random expectations ($P < 0.01$; Table 1). According to the estimates of the relative contribution of environmental variables to the *Dianthus broteri* complex model (Table 2), mean monthly *PET* of coldest quarter was the variable contributing the highest explanatory power (36.6%), followed by mean monthly *PET* of driest quarter (13.5%)

and mean monthly *PET* of wettest quarter (9.5%). For 2x, $4x_{pool}$ and 6x cytotypes, mean monthly *PET* of coldest quarter was also an important predictor (Table 2) but different variables contributed to model each cytotype: *BIO18* in diploids and the two tetraploid lineages, mean monthly *PET* of warmest quarter in tetraploids and hexaploids, and *BIO9* in dodecaploids. These models were robust to sample size. Once again, models developed with the reduced dataset generated similar suitability maps but new regions of low suitability (< 0.3) appeared generally northward (Fig. S3). The explanatory power of the model predictors was mostly congruent (Table S2) but not all AUC values were significantly higher than those generated by null models (Table S1).

Niche comparison between cytotypes

Extensive variation in the ecological niche breadth of cytotypes was detected (Table 1; Fig. 2c, d). The tetraploids showed the broadest distribution of suitable habitats, followed by diploids, hexaploids and, finally, dodecaploids, which had the narrowest distribution of suitability, about 25 times smaller than $4x_{pool}$. Even considering each tetraploid lineage ($4x_s$ and $4x_e$) independently, they had greater niche breadths than 6x and 12x (Table 1). In addition, the niche overlap between cytotypes correlated positively with the niche breadth ($r^2 = 0.43$; $P < 0.05$; $n = 12$). Overall, the $4x_{pool}$ cytotype presented the highest overlapping niche with all the remaining cytotypes, followed again by diploids, hexaploids and, finally, dodecaploids. As expected, 2x and $4x_{pool}$ cytotypes, which had the broadest niches, showed the greatest overlap ($D = 0.53$; Table 3). This overlap increased when only the southern tetraploid lineage was considered ($D = 0.65$; Table 3). Moreover, the $4x_e$ lineage largely overlapped with the 6x cytotype ($D = 0.36$; Table 3). However, it is notable that the two cytotypes with more distinct and narrower niches (6x and 12x) did not overlap at all (Table 3).

In general, the environmental niches of each cytotype/lineage were different ($P < 0.05$) for every pairwise combination in equivalency tests (Table 3), except for 2x-$4x_s$. Whereas the comparison between 2x and $4x_{pool}$ niches gave a significant similarity in both directions ($P < 0.05$), the remaining similarity tests revealed that niche differences were not due to the geographically available environmental conditions (i.e. the cytotypes are more divergent than expected based on their potential available ranges; Table 3). These patterns were mainly confirmed using the reduced dataset but with lower statistical significance (Table S3). The ecological niches of 2x and $4x_{pool}$ were not only similar but also equivalent, $4x_{pool}$ and 12x ones were similar and $4x_e$-6x and $4x_s$-12x showed

equivalency. All niche breadth and almost all overlap values were lower than in the complete dataset.

Environmental and phylogenetic niche conservatism

Accordingly, the divergence tests for the environmental gradients showed significant divergence of the 12x cytotype in the PC1 axis and the same pattern for the 6x cytotype in the PC2 axis (Table 3). Whereas 2x-12x and 6x-12x niche comparisons showed divergence in both axes, 2x and $4x_{pool}$ niches appeared to be conserved (Table 3). Nevertheless, both southern and eastern 4x lineages diverged in the two main PCA axes compared to the rest of cytotypes and, interestingly, to each other. As an exception, 2x-$4x_s$ niche comparison showed conservatism in the PC2 due to their niche equivalency and high overlap. These results were consistent with those obtained using the reduced dataset. In the PC1, the 12x cytotype showed divergence except regarding the 6x and the $4x_s$. In the PC2, the 6x niche diverged significantly from the rest. The 2x-$4x_{pool}$ comparison presented again conservatism in both axes (Table S3). These analyses confirmed that $4x_s$ and $4x_e$ niches did not overlap and largely diverged from each other.

Finally, we found significant phylogenetic signal for the two axes ($K \geq 1$, $P < 0.01$) suggesting PNC along the environmental space (Fig. 4). The PC1 and PC2 variation better fitted the OU5 model (i.e. the one considering a scenario with five niche optima, corresponding to the four ploidies including the two independent origins of the tetraploids) with AICc weights > 0.80 (Fig. 4). Congruently, the OU5 was the best fitting model for the complete environmental space, with an AICc weight virtually of one (null for the rest of models), supporting not just PNC at population scale (i.e. within lineage) but also niche divergence between polyploid lineages including the two tetraploids.

Discussion

Ecological drivers of cytotype distributions

Overall, our niche models suggested that the distribution of *D. broteri* is constrained by environmental variables related to temperature and drought stresses (potential evapotranspiration in the driest and warmest quarters) imposed by the Mediterranean climate, even more pronounced under climate change predictions (Gasith & Resh, 1999; Giorgi & Lionello, 2008). The realized range of this complex (i.e. realized niche) was similar to the estimated potential habitat (i.e. fundamental niche), suggesting that the current range is constrained by ecological/physiological tolerances (Guisan & Thuiller,

2005; McGill *et al.*, 2006) more than historical or dispersal limitations (Lobo *et al.*, 2010; Glennon *et al.*, 2014). Additionally, edaphic properties played a key role driving *D. broteri* distribution and lineage divergence. Soil texture (i.e. sand content) and pH were the most relevant variables. Whereas pH is known as a key predictor for the occurrence of plant species, since it affects the availability of nutrients and phytotoxic metals (Wagner *et al.*, 2017), soil texture mainly influences the water holding capacity and therefore it is important in the adaptation to Mediterranean dry biomes (Saxton & Rawls, 2006; Padilla & Pugnaire, 2007).

Autopolyploidy, niche evolution and competitive interactions

We observed consistent environmental gradients fostering niche evolution in *D. broteri* complex, and we identified two patterns of polyploid niche shifts within this series. Whereas we found evidence of niche expansion in tetraploids (related to diploids), the higher polyploids demonstrated a trend to occupy specialized niches in narrow and stressful habitats (i.e. niche novelty *sensu* Marchant *et al.*, 2016). Diploid and tetraploid niches were similar but the tetraploid cytotype showed a more widespread range according to its wider niche breadth. Although a similarity test between these *D. broteri* cytotypes was previously performed by Glennon *et al.* (2014) and gave congruent results, it failed to find differences between niches and the overlap was remarkably higher (0.70 vs 0.53). Such differences could be due to the inclusion of topo-edaphic factors as well as other more meaningful climatic variables for Mediterranean plants (e.g. *PET*s, climatic moisture index or precipitation of warmest quarter; Detto *et al.*, 2006; Dubuis *et al.*, 2013).

However, the niche overlap pattern is clearly different when each 4x lineage was considered independently. We found niche equivalence and the highest overlap in the comparison 2x-4x_s but different, non-overlapping and divergent niches between 2x and 4x_e. The differences in genetic isolation and divergence of both tetraploid lineages from diploids (Balao *et al.*, 2010) might help to explain this pattern. Nevertheless, diploids and tetraploids do not actually coexist (absence of mixed-cytotype populations) and therefore other ecological factors may have driven the geographic segregation. In this context, the disjunct distribution of diploids (Balao *et al.*, 2009) might be due to competitive exclusion in arid localities by southern tetraploids (4x_s), whose phenotypic changes (Balao *et al.*, 2011a) could help colonize competitive environments as suggested for the southern Spain populations of *Brachypodium distachyon* tetraploids (Rey *et al.*, 2017). Another non-

exclusive explanation for this segregation pattern relies on a differentiation in the biotic niche (Wisz *et al.*, 2013). Divergence in pollinator spectra and/or visit frequency have been found in other polyploids (Kennedy *et al.*, 2006; Thompson & Merg, 2008; Husband *et al.*, 2016). In *D. broteri*, the reproductive biology of 2x, 4x and 6x cytotypes is unknown but the dodecaploids have showed an extremely specialized pollination (Balao *et al.*, 2011b). In addition, floral changes associated with ploidy in *D. broteri* (Balao *et al.*, 2011a) may influence pollinator preference and reproductive isolation (Segraves & Thompson, 1999).

The higher polyploids (6x and 12x cytotypes) have diverged towards environmental margins, and occur in specialized and mostly non-overlapping niches with regard to the other cytotypes. This niche divergence pattern is similar to that found in a broad survey within the tribe Potentilleae of the Rosaceae family, which included more than 100 species and six different ploidy levels (Brittingham *et al.*, 2018). In *D. broteri*, 6x and 12x niches were characterized by the most extreme conditions (high temperatures and scarce water availability) of the Mediterranean climate. The hexaploid cytotype inhabits a semi-arid Mediterranean area in SE Spain notable for the absence of seasonality, with a low precipitation and high temperatures, and *PET*s limiting the wettest periods (López-Bermúdez, 1990). Furthermore, the 6x niche was also characterized by eroded, unfertile (i.e. low organic carbon content) and basic soils as expected in arid or semi-arid habitats (Barea *et al.*, 2011). These features constrain plant growth and cause functional specialization and diversity (Rundel *et al.*, 2016). Interestingly, hexaploids also share a portion of the described environmental niche with eastern tetraploids (mostly soils with high pH and harsh climatic conditions in the wettest quarter), which were probably involved in their origin by hybridization with southern diploids (Balao *et al.*, 2010). The origin of the dodecaploids is unclear but, based on the molecular and genome size data, it seems that they have evolved largely independently of the other cytotypes (Balao *et al.*, 2009; Balao *et al.*, 2010). This 12x cytotype has acquired adaptations to allow it to survive the harsh summer (with low precipitations and high temperatures) in the Doñana National Park area (S Spain; Zunzunegui *et al.*, 2005), where it is currently distributed. In addition, this area is characterized by sandy soils with high bulk density (i.e. paleodunes) which inherently have a low available water content (McNabb *et al.*, 2001; Obia *et al.*, 2016). These soil properties have major effects on plant growth (Maun, 1994; Place *et al.*, 2008; Tracy *et al.*, 2013).

Phylogenetic niche conservatism and recurrent polyploid origins

Evolutionary models for individual environmental axes and for the global environmental space indicated that divergent selection has driven niche evolution in polyploids; this supports the 'minority cytotype exclusion' (MCE) theory (Levin, 1975) and/or subsequent competitive interactions (Laport *et al.*, 2013; Rey *et al.*, 2017). In fact, the presence of triploid individuals in a low proportion within a diploid population of this polyploid complex probably reveals an unstable or intermediate autopolyploid evolutionary step (Husband, 2004). Moreover, our results highlight the importance of multiple origins in polyploid niche evolution. The enhanced ecological tolerances of tetraploids, encompassing the most diverse environmental conditions, could be partially explained by their two largely unrelated lineages (Balao *et al.*, 2010). As a consequence, each 4x origin has colonized a different niche space, as confirmed by their completely different and non-overlapping distributions. In contrast, 6x and 12x cytotypes have evolved in distinct single events, and show notable genetic relatedness within cytotypes (Balao *et al.*, 2010), which may have influenced their distributions and specialized ecological tolerances. It is also noteworthy that these high polyploids showed increased epigenetic marks (i.e. cytosine DNA methylation) but also higher epigenetic variability (Alonso *et al.*, 2016), which could be crucial for adaptation and survival in extreme Mediterranean habitats (Mirouze & Paszkowski, 2011; Balao *et al.*, 2018).

In the *D. broteri* complex, tetraploids are found in a broad habitat range because they were capable of exploiting new niches (i.e. ecological release with niche expansion) and, as a consequence, higher polyploids have had to adapt to more extreme, and necessarily smaller, ecological niches (i.e. niche contraction and specialization). Theodoridis *et al.* (2013) and Thompson *et al.* (2014) provided evidence for the ecological superiority of higher cytotypes in *Primula* sect. *Aleuritia* and *Chamerion angustifolium*, respectively. Furthermore, in *Galax urceolata* complex, tetraploids have experienced niche contraction and divergence with respect to the ancestral wide niche of diploids (Gaynor *et al.*, 2018). Contrariwise, in cases where low ploidy cytotypes were unable to expand to occupy their full potential niche (Lowry & Lester, 2006), and/or multiple polyploid origins occur, the higher levels could experience niche expansion, as in *Aster amellus* (Münzbergová *et al.*, 2013), *Claytonia perfoliata* (McIntyre, 2012), *Larrea tridentata* (Laport *et al.*, 2016) or *Senecio carniolicus* (Sonnleitner *et al.*, 2016). These are dynamic systems with frequent coexistence in mixed-ploidy populations (Kolář *et al.*,

2017), and where the apparent competitive superiority of the higher cytotypes may be enhanced by their recurrent polyploid formation and the alleged unfilled niches of lower ploidies.

To sum up, we proposed to unify the multiple origins of polyploids, competitive interactions and the niche filling theory into a single framework, which should be able to explain and predict any niche evolution pattern in polyploid complexes.

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Author contributions

F.B. and E.M.N. conceived the idea; J.L.J. and F.B. gathered the data, designed and performed the analyses; J.L.J. and F.B. drafted the text; all authors interpreted the results, provided corrections to manuscript drafts and discussed ideas within it.

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Figure legends

Fig. 1 Hypothesized conceptual framework of autopolyploid niche evolution. Grey circles represent the available niche space and black circles indicate the realized niche of the cytotypes. Continuous and dashed arrows designate polyploidization events and recurrent origins, respectively.

Fig. 2 Representation of the principal component analysis (PCA-env) obtained for GBIF and cytotyped locations together of *Dianthus broteri*, using non-collinear variables and background points. It represents the environmental spaces of the niches in two main axes (greater inertia explained). Panel (a) presents the distribution of the selected variables loading on these axes (see Table 2 for a description of these variables). Panel (b) shows the niches of the four cytotypes (ellipses encompass occurrence points) in the two main axes. The tetraploid level was divided into its two lineages. Panels (c) and (d) are a breakdown of (b) by the axes, presenting the environmental range of the groups as violin plots. In these plots, white circles represent the median, thick black bars correspond to the interquartile range and thin bars show the 95% confidence interval.

Fig. 3 Ecological niche models for *Dianthus broteri* species (all populations) and for its four cytotypes separately, using GBIF and cytotyped occurrences together. The tetraploid level was divided into its two lineages. The maps highlight geographic space with environmental suitability using increasing hot colors. Grey areas indicate that they are not suitable (value of 0) and dark red areas indicate the maximum suitability (value of 1.0). All maps represent the Iberian Peninsula and the Balearic Islands. Black dots designate presence locations.

Fig. 4 Phenograms for the two main environmental axes constructed with data corresponding to the *Dianthus broteri* population phylogeny (n = 25) from Balao *et al.* (2010). The tables on the upper left corners summarize each model performed with AICc values and their weights. Labels on the right margin indicate the names of the populations. Asterisks mark the eastern tetraploid clade.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Principal component analysis (PCA-env) for *D. broteri* cytotyped locations, representing each niche and the selected variables.

Fig. S2 Suitability maps of *D. broteri* complex representing ecological niche models using cytotyped occurrences.

Fig. S3 Suitability maps of *D. broteri* 4x cytotype representing ecological niche models using GBIF and cytotyped occurrences together and only the cytotyped ones.

Table S1 Evaluation of ecological niche models and niche breadth values using cytotyped occurrences

Table S2 Variable contribution to the construction of the models using only the cytotyped occurrences

Table S3 Ecological niche comparisons for *D. broteri* ploidies using only the cytotyped records

Methods S1 Predictor layers can be downloaded from public databases. All R scripts for niche comparison tests, habitat suitability models and PNC analyses are available at a GitHub repository DOI: 10.5281/zenodo.2388457 (https://github.com/fbalao/envdianthus/tree/v1.0).

Tables

Table 1 Summary of the evaluation of ecological niche models by their AUC and null model scores along with the niche breadth metric. Results correspond to inputs of GBIF and cytotyped occurrences together.

D. broteri lineages	*n*	AUC*	Null model AUC	Niche breadth
2x	29	0.987	0.838	0.0924
$4x_{pool}$	52	0.957	0.778	0.2341
$4x_{s}$	32	0.988	0.853	0.0813
$4x_{e}$	20	0.989	0.867	0.1164
6x	21	0.987	0.873	0.0386
12x	48	0.999	0.787	0.0095
All populations	150	0.974	0.683	-

n, number of occurrences (populations) used for modelling the distributions.

*All models have a significantly higher AUC value when compared to their null distribution ($P < 0.01$) based on 99 repetitions (only the highest null model score presented).

Table 2 Percentage of variable contribution to the models using GBIF and cytotyped occurrences together. The values correspond to the Permutation Importance analysis from MaxEnt. For each *Dianthus broteri* cytotype (and both tetraploid origins), the three variables with the highest contribution are marked in bold

Variable	Description	All populations	2x	$4x_{pool}$	$4x_s$	$4x_e$	6x	12x
BIO3	Isothermality	2.8	0.2	0.4	0.2	0.0	2.5	8.2
BIO5	Max temperature of warmest month	0.5	2.9	0.0	1.5	0.3	0.0	3.0
BIO8	Mean temperature of wettest quarter	2.3	1.0	0.4	2.2	0.0	0.0	0.0
BIO9	Mean temperature of driest quarter	0.2	0.0	0.0	0.0	0.0	0.0	**37.0**
BIO12	Annual precipitation	0.3	0.6	0.5	0.3	0.1	3.6	0.7
BIO18	Precipitation of warmest quarter	8.9	**31.8**	2.0	**71.5**	**32.6**	0.0	0.5
PETColdestQuarter	Mean monthly PET of coldest quarter	**36.6**	**10.0**	**24.7**	**5.2**	0.0	**37.0**	0.4
PETDriestQuarter	Mean monthly PET of driest quarter	**13.5**	8.2	1.1	0.0	0.0	**10.0**	0.0
PETWarmestQuarter	Mean monthly PET of warmest quarter	4.3	0.9	**23.3**	1.0	**48.3**	**44.6**	2.3
PETWettestQuarter	Mean monthly PET of wettest quarter	9.4	**36.8**	1.3	1.6	0.0	0.6	0.0
climaticMoistureIndex	A metric of relative wetness and aridity	0.5	3.0	**20.6**	4.1	**17.9**	0.1	**27.7**
topoWet	SAGA-GIS topographic wetness index	4.4	0.8	1.1	0.4	0.6	0.0	0.0
tri	Terrain roughness index	1.3	0.9	1.8	0.2	0.1	1.1	**13.0**

AWC	Available soil water capacity	0.6	0.0	1.2	0.0	0.0	0.0	0.0
BLDFIE	Bulk density (fine earth) in kg / cubic-meter	0.4	0.0	0.2	0.0	0.0	0.1	0.0
CECSOL	Cation exchange capacity of soil in cmolc/kg	1.5	0.4	1.2	1.2	0.0	0.0	0.2
ORCDRC	Soil organic carbon content (fine earth fraction) in g per kg	0.1	0.0	0.1	0.3	0.0	0.0	4.7
PHIHOX	Soil pH x 10 in H_2O	2.9	2.2	16.2	**6.5**	0.0	0.3	2.2
SNDPPT	Sand content (50-2000 micro meter) mass fraction in %	**9.5**	0.4	4.0	3.8	0.0	0.0	0.1

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Table 3 Ecological niche comparisons for *Dianthus broteri* using cytotyped and GBIF records. Columns correspond to the different tests performed in the two main PCA-env axes: overlapping, similarity (all the comparisons in both directions), equivalency and environmental divergence (derived from McCormack *et al.*, 2010)

D. broteri lineages			Niche similarity			Environmental divergence test	
a	*b*	Niche overlap (*D*)	$a \rightarrow b$	$b \rightarrow a$	Niche equivalency	AXIS 1	AXIS 2
2x	$4x_{pool}$	0.5296	Similar*	Similar*	Different*	C	C
	$4x_s$	0.6492	-	-	ns	D**	C
	$4x_e$	0.0848	ns	ns	Different*	D**	D**
	6x	0.0436	ns	ns	Different*	C	D**
	12x	0.1525	ns	ns	Different*	D**	D**
$4x_{pool}$	6x	0.1452	ns	ns	Different*	C	D**
	12x	0.1292	ns	ns	Different*	D**	C
$4x_s$	$4x_e$	0.0172	ns	ns	Different*	D**	D**
6x	$4x_s$	0.0002	ns	ns	Different*	D**	D**
	$4x_e$	0.3648	ns	ns	Different*	D**	D**
	12x	0	ns	ns	Different*	D**	D**
12x	$4x_s$	0.2643	ns	ns	Different*	D**	D**
	$4x_e$	0	ns	ns	Different*	D**	D**

ns, not significantly different/equivalent

*The ecological niches are significantly ($P < 0.05$) more *similar* or *different* than expected by random.

**The ecological niches are significantly ($P < 0.05$) different or show *divergence* according to Student's *t*-test.

C, conservatism.

D, divergence.