

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

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4 Javier López-Jurado*, Enrique Mateos-Naranjo, Francisco Balao

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6 Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de
7 Sevilla, Apdo. 1095, E-41080 Sevilla, Spain

8

9 *Corresponding author.

10 **E-mail address:** javlopez@us.es

11 **Tel.:** +34 95 4552763

12

13 **ORCID**

14 Javier López-Jurado: <https://orcid.org/0000-0002-6354-0800>

15 Enrique Mateos-Naranjo: <https://orcid.org/0000-0001-6276-5664>

16 Francisco Balao: <https://orcid.org/0000-0003-2104-3846> (Twitter: [@fbalao](https://twitter.com/fbalao))

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27 **Summary**

- 28 • Niche evolution in plant polyploids remains controversial and evidence for alternative
29 patterns has been reported. Using the autopolyploid *Dianthus broteri* complex (2x,
30 4x, 6x and 12x) as a model, we aimed to integrate three scenarios, competitive
31 exclusion, recurrent origins of cytotypes and niche filling, into a single framework of
32 polyploid niche evolution. We hypothesized that high polyploids would tend to evolve
33 towards extreme niches when low ploidy cytotypes have nearly filled the niche space.
- 34 • We used several ecoinformatics and phylogenetic comparative analyses to quantify
35 differences in the ecological niche of each cytotype and to evaluate alternative models
36 of niche evolution.
- 37 • Each cytotype in this complex occupied a distinct ecological niche. The distributions
38 were mainly constrained by soil characteristics, temperature and drought stress
39 imposed by the Mediterranean climate. Tetraploids had the highest niche breadth and
40 overlap due to their multiple origins, while the higher ploidy cytotypes were found in
41 different, restricted, non-overlapping niches. Niche evolution analyses suggested a
42 scenario with one niche optimum for each ploidy, including the two independent
43 tetraploid lineages.
- 44 • Our results suggest that the fate of nascent polyploids could not be predicted without
45 accounting for phylogenetic relatedness, recurrent origins or the niche occupied by
46 ancestors.

47 **Key words**

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

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55 **Introduction**

56 Polyploidization (i.e. the process of acquiring more than two complete sets of
57 chromosomes) is one of the major driving forces in plant evolution (Wendel, 2000; Otto,
58 2007; Soltis *et al.*, 2015). Neopolyploid establishment may be constrained by a
59 frequency-dependent mating disadvantage compared to their diploid parents, called
60 ‘minority cytotype exclusion’ (MCE; Levin, 1975). For this reason, polyploids need to
61 outcompete their parents or ecologically differentiate from them (‘niche escape’). The
62 genome duplication may drive phenotypic changes (Balao *et al.*, 2011a; Laport *et al.*,
63 2016; Rey *et al.*, 2017), which can lead to shifts in environmental tolerances and so
64 promote increased competitive abilities or subsequent habitat displacement (i.e. niche
65 divergence with expansion or contraction; Manzaneda *et al.*, 2012; Thompson *et al.*,
66 2014; Visger *et al.*, 2016).

67 Despite several decades of studies and recent attempts to unify theoretical
68 frameworks (Parisod & Broennimann, 2016), niche evolution in polyploids remains
69 controversial and evidence for several alternative patterns has been found (Glennon *et al.*,
70 2014; Visser & Molofsky, 2015; Marchant *et al.*, 2016). Niche expansion in
71 neopolyploids may be helped by the lack of selective constraints on duplicate genes and
72 other novel genetic changes that allow the evolution of new functions (Doyle *et al.*, 2008;
73 Leitch & Leitch, 2008; Wendel, 2015). However, the opposite pattern of niche evolution,
74 niche contraction, has also been suggested (Theodoridis *et al.*, 2013; Kirchheimer *et al.*,
75 2016). On this latter scenario, polyploids would move to narrower and marginal niches
76 with specific ecological conditions, leading to habitat specialization and promoting new
77 habitat colonization (especially at the extreme edges of environmental gradients; Buggs
78 & Pannell, 2007; Boulangeat *et al.*, 2012). Finally, the absence of ecological niche
79 differentiation between cytotypes could be explained by Phylogenetic Niche
80 Conservatism (PNC), the tendency for lineages to preserve ancestral niche-related traits
81 (Crisp & Cook, 2012), or short-range dispersal (Duchoslav *et al.*, 2010) or small
82 differences in relative competitive abilities (Bulleri *et al.*, 2016). One would expect PNC
83 to be more common in autopolyploids (polyploids formed by within-species genome
84 duplication) because of their higher genetic relatedness to their progenitors (Burns &
85 Strauss, 2011; Glennon *et al.*, 2014), although this theory has scarcely been tested (but
86 see Arrigo *et al.*, 2016).

87 An explanation for the divergence patterns could be that the niche evolution in a
88 polyploid is ultimately limited by niche filling (Tanentzap *et al.*, 2015), which may act at
89 lineage level (based on its origin) more than merely at cytotype level. The first formed
90 polyploids would fill the unoccupied niche space to avoid competition with their ancestor.
91 In this initial step, nascent polyploids would have a high probability of niche expansion.
92 This fact prevents subsequent higher cytotypes from access to such niches, so newcomers
93 must diverge to find available niches for themselves. Eventually, niche expansion would
94 be less likely since higher ploidies encounter limits to their ecological tolerances (Fig. 1;
95 Araújo *et al.*, 2013). Additionally, multiple origins of polyploids (i.e. recurrent formation)
96 are the rule (Soltis & Soltis, 1999) and would cause an increased genetic, biochemical
97 and physiological diversity, conferring the ability to colonize new environments or
98 achieve a broader geographic range (Treier *et al.*, 2009; McIntyre, 2012; Karunaratne *et*
99 *al.*, 2018). In this case, local adaptation within cytotype would also play an important role
100 in its ecological differentiation (Maherali *et al.*, 2009; Ramsey, 2011; McIntyre & Strauss,
101 2017). Therefore, patterns of niche evolution in polyploid complexes are likely to be
102 dynamic and non-exclusive.

103 A study of a large autopolyploid series, that avoids the effects of hybridization
104 (typical of allopolyploids; Spoelhof *et al.*, 2017), may provide insights into the different
105 processes driving polyploid niche evolution. Such insights may be enhanced if we
106 consider information on the phylogenetic relatedness among populations (i.e. the
107 phylogenetic signal in environmental traits). *Dianthus broteri* (Caryophyllaceae) is an
108 excellent system to investigate these underlying causes of niche evolution. This Iberian
109 endemic complex presents the most extensive autopolyploid series for the genus, with 2x,
110 4x, 6x and 12x cytotypes that very rarely coexist at the same location. In fact, only one
111 mixed-ploidy population with diploid individuals and a few triploids has been described
112 in southwestern Portugal (Balao *et al.*, 2009). While 6x and 12x cytotypes have
113 independently evolved by single events, the tetraploids have been recurrently originated
114 by two polyploidization events (Balao *et al.*, 2010). Furthermore, the four cytotypes
115 present differentiated geographic distributions (Balao *et al.*, 2009). The diploids occur in
116 two disjunct areas (south of Portugal and the mountainous southeast of Spain) whilst the
117 4x cytotype inhabits the broadest geographical range, with a southern lineage (4x_s)
118 distributed from the southwest of Portugal to the south of Spain, and an eastern lineage
119 (4x_e) that occurs in the east of Spain. In contrast, the higher ploidies (6x and 12x

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

127 In the present study, we used several ecoinformatics and phylogenetic comparative
128 analyses (Methods S1) to shed light on the niche evolution underlying the rapid radiation
129 by autopolyploidy in the extant cytotypes of *Dianthus broteri* complex (Balao *et al.*, 2009,
130 2010). We hypothesize that the higher cytotypes have adapted to more specialized
131 environments whereas the lower cytotypes are distributed encompassing a wider
132 environmental range. Additionally, the multiple origins of tetraploids would have
133 contributed to the development of a broad niche (Fig. 1). In alternative scenarios, the high
134 polyploids would just retain the niche of lower cytotypes, reflecting PNC, and the lineages
135 recurrently originated would share the ecological niche conditions. Thus, we address the
136 following specific questions: Is the current cytotype distribution a consequence of PNC
137 or niche divergence? Are the higher polyploids occupying extreme niches because the
138 lower ploidies have nearly filled the niche space? And have the multiple origins of the
139 tetraploids influenced their current ecological niche locations?

140 **Materials and Methods**

141 Occurrence data and cytotype distribution

142 Locality information was collected from Global Biodiversity Information Facility (GBIF)
143 using the package ‘*rgbif*’ (Chamberlain *et al.*, 2017) in R software vers. 3.4.2 (R Core
144 Team, 2017). We restricted the dataset to accurately georeferenced locations with known
145 herbarium vouchers. We added to this information a few additional localities from our
146 personal observations and 29 populations whose ploidy was confirmed by flow cytometry
147 (Supporting Information Table S1). Duplicate occurrences were removed, and locally
148 dense sampling was reduced by thinning the records to one per 1 km² grid cell size
149 resulting in 150 localities in total. We assigned the ploidy for all GBIF records based on
150 the clearly delimited distribution areas of the different cytotypes, which occurred as
151 monocytotypic populations (Balao *et al.*, 2009), and the morphological differences of the
152 vouchers using an ad-hoc single-access key based on Balao *et al.* (2011a). For robustness,

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

155 Environmental data

156 To obtain a detailed description of the environmental niche characteristics for the
157 locations of the *D. broteri* cytotypes, we used a large up-to-date and high-resolution set
158 of predictor layers from four different databases. We selected variables with a likely
159 relevance to the ecological and physiological conditions and constraints that determine
160 the niches of terrestrial plant species: (1) 19 bioclimatic variables from CHELSA database
161 (Karger *et al.*, 2017) at ~1 km² resolution, (2) a selection of 11 climatic and 2 topographic
162 continuous variables from ENVIREM database (Title & Bemmels, 2018) at ~1 km²
163 resolution, (3) 7 relevant edaphic variables from SoilGrids database (Hengl *et al.*, 2017)
164 at a resolution of 250 m² and (4) the altitude information from WorldClim (Fick &
165 Hijmans, 2017). We removed highly correlated environmental variables in the datasets
166 using the variance inflation factor (VIF) with a threshold of 10 (indicating collinearity).
167 We finally obtained for the complete dataset an equitable set of covariates from the four
168 sources used (6 bioclimatic, 5 environmental plus the 2 topographic and 6 edaphic
169 variables; see Fig. 2a). In the reduced dataset, the selected environmental variables were
170 similar with just two different variables (Table S2; Fig. S1).

171 Ecological Niche Modelling

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

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

187 Niche comparisons: breadth, overlap, equivalency and similarity tests

188 To compare the environmental niche of the different ploidies (including the 4x lineages),
189 we calculated the kernel-smoothing densities of each occurrence data along
190 environmental axes from a Principal Component Analysis (PCA-env; Broennimann *et*
191 *al.*, 2012). For the background, we extracted the environmental data from 10,000 spatially
192 thinned random localities within a buffer of 150 km surrounding presence points. We
193 estimated Levins' measure of niche breadth (Levins, 1968) for each cytotype using the
194 'ENMTools' package (Warren *et al.*, 2010) in R. Furthermore, we estimated the
195 ecological niche overlap between them and performed the niche equivalency test using
196 the Schoener's *D* statistic (Schoener, 1968) in the 'ecospat' R package (Di Cola *et al.*,
197 2017). Niche equivalency tests compared the observed overlap of *D* values to a null
198 distribution using 100 replicates and an environmental grid resolution of 500 × 500 pixels.
199 In order to refine the niche comparisons, we also performed niche similarity tests to every
200 pair of non-equivalent niches in both directions (Broennimann *et al.*, 2012). We compared
201 the observed overlap value between two occurrence groups to a null distribution of 100
202 overlap values of one of them and a randomly simulated niche in the available
203 environmental range of the other niche. Finally, as a validation approach, we ran all niche
204 comparison tests using the reduced dataset.

205 Environmental and phylogenetic niche conservatism

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

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

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

234 **Results**

235 Distribution along environmental gradients

236 The environmental variation within and among ploidies in *D. broteri* was mainly
237 represented in the first two PCA axes (Fig. 2b-d), explaining respectively 28.2% and
238 21.3% of the total variation in the environmental space. These PCA axes summarized two
239 environmental gradients that constrained the cytotype distributions. The PC1 axis was
240 related to seasonal environmental variables characteristic of the Mediterranean climate
241 and to soil bulk density (*BLDFIE*) and available water capacity (*AWC*). The 12x cytotype
242 occupied an extreme position in this axis (PC1; Fig. 2c), corresponding to localities with
243 harsh dry summers characterized by low precipitation of warmest quarter (*BIO18*) and
244 *AWC* in soils, and a high topographic wetness index (*topoWet*) and potential
245 evapotranspiration (*PET*) in driest and warmest quarters. Furthermore, this cytotype was
246 also associated to warm summers (high maximum temperature of warmest month, *BIO5*,
247 and mean temperature of driest quarter, *BIO9*) and located in flat terrains (low terrain
248 roughness index, *tri*) with high *BLDFIE* (predominantly sandy soils). The PC2 axis
249 mirrored a general aridity gradient (in terms of annual environmental means and with

250 severe conditions of temperature and rainfall affecting the wettest period of the year). In
251 this case, the axis mainly constrained the distribution of the 6x cytotype (Fig. 2d),
252 showing the lowest values of annual precipitation (*BIO12*) and the metric of relative
253 wetness and aridity (*climaticMoistureIndex*) and the highest ones of *topoWet* and *PET* in
254 the wettest quarter (dry climate conditions). The warm climate conditions predominant in
255 the niche of this cytotype were characterized by low isothermality (*BIO3*) and high *BIO5*
256 and mean temperature of wettest quarter (*BIO8*). Moreover, the hexaploids inhabit poor
257 (low organic carbon content, *ORCDRC*), not sandy (low sand content, *SNDPPT*) and
258 alkaline (high pH, *PHIHOX*) soils. In this environmental space, 2x and 4x_{pool} (4x_e + 4x_s)
259 cytotypes were not clearly differentiated from each other. Whereas pooled tetraploid
260 lineages encompass the complete range of the environmental conditions, diploids are
261 located in rich, sandy, acid soils with more benign characteristics (the lowest position in
262 the PC2 axis). However, the two tetraploid lineages (4x_s and 4x_e) were environmentally
263 distant from each other. The conditions of the southern tetraploids (4x_s) resembled those
264 of 2x and 12x cytotypes (suffering harsher summers with low values in both axes; Fig.
265 2b-d), whereas the eastern tetraploids (4x_e) occur in the right extreme of the PC1 axis and
266 near the 6x populations in the PC2 axis (Fig. 2b-d; milder summers, with non-sandy and
267 watered soils, but several arid conditions throughout the year). These results were robust
268 as similar ones were found using the reduced dataset (Fig. S1). In this last analysis, the
269 two axes described the same environmental gradients but they maximized the
270 environmental distance between cytotypes/lineages.

271 Environmental suitability and hotspots

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

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

292 Niche comparison between cytotypes

293 Extensive variation in the ecological niche breadth of cytotypes was detected (Table 1;
294 Fig. 2c, d). The tetraploids showed the broadest distribution of suitable habitats, followed
295 by diploids, hexaploids and, finally, dodecaploids, which had the narrowest distribution
296 of suitability, about 25 times smaller than 4x_{pool}. Even considering each tetraploid lineage
297 (4x_s and 4x_e) independently, they had greater niche breadths than 6x and 12x (Table 1).
298 In addition, the niche overlap between cytotypes correlated positively with the niche
299 breadth ($r^2 = 0.43$; $P < 0.05$; $n = 12$). Overall, the 4x_{pool} cytotype presented the highest
300 overlapping niche with all the remaining cytotypes, followed again by diploids,
301 hexaploids and, finally, dodecaploids. As expected, 2x and 4x_{pool} cytotypes, which had
302 the broadest niches, showed the greatest overlap ($D = 0.53$; Table 3). This overlap
303 increased when only the southern tetraploid lineage was considered ($D = 0.65$; Table 3).
304 Moreover, the 4x_e lineage largely overlapped with the 6x cytotype ($D = 0.36$; Table 3).
305 However, it is notable that the two cytotypes with more distinct and narrower niches (6x
306 and 12x) did not overlap at all (Table 3).

307 In general, the environmental niches of each cytotype/lineage were different ($P <$
308 0.05) for every pairwise combination in equivalency tests (Table 3), except for 2x-4x_s.
309 Whereas the comparison between 2x and 4x_{pool} niches gave a significant similarity in both
310 directions ($P < 0.05$), the remaining similarity tests revealed that niche differences were
311 not due to the geographically available environmental conditions (i.e. the cytotypes are
312 more divergent than expected based on their potential available ranges; Table 3). These
313 patterns were mainly confirmed using the reduced dataset but with lower statistical
314 significance (Table S3). The ecological niches of 2x and 4x_{pool} were not only similar but
315 also equivalent, 4x_{pool} and 12x ones were similar and 4x_e-6x and 4x_s-12x showed

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

318 Environmental and phylogenetic niche conservatism

319 Accordingly, the divergence tests for the environmental gradients showed significant
320 divergence of the 12x cytotype in the PC1 axis and the same pattern for the 6x cytotype
321 in the PC2 axis (Table 3). Whereas 2x-12x and 6x-12x niche comparisons showed
322 divergence in both axes, 2x and 4x_{pool} niches appeared to be conserved (Table 3).
323 Nevertheless, both southern and eastern 4x lineages diverged in the two main PCA axes
324 compared to the rest of cytotypes and, interestingly, to each other. As an exception, 2x-
325 4x_s niche comparison showed conservatism in the PC2 due to their niche equivalency and
326 high overlap. These results were consistent with those obtained using the reduced dataset.
327 In the PC1, the 12x cytotype showed divergence except regarding the 6x and the 4x_s. In
328 the PC2, the 6x niche diverged significantly from the rest. The 2x-4x_{pool} comparison
329 presented again conservatism in both axes (Table S3). These analyses confirmed that 4x_s
330 and 4x_e niches did not overlap and largely diverged from each other.

331 Finally, we found significant phylogenetic signal for the two axes ($K \geq 1$, $P <$
332 0.01) suggesting PNC along the environmental space (Fig. 4). The PC1 and PC2 variation
333 better fitted the OU5 model (i.e. the one considering a scenario with five niche optima,
334 corresponding to the four ploidies including the two independent origins of the
335 tetraploids) with AICc weights > 0.80 (Fig. 4). Congruently, the OU5 was the best fitting
336 model for the complete environmental space, with an AICc weight virtually of one (null
337 for the rest of models), supporting not just PNC at population scale (i.e. within lineage)
338 but also niche divergence between polyploid lineages including the two tetraploids.

339 **Discussion**

340 Ecological drivers of cytotype distributions

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

348 2005; McGill *et al.*, 2006) more than historical or dispersal limitations (Lobo *et al.*, 2010;
349 Glennon *et al.*, 2014). Additionally, edaphic properties played a key role driving *D.*
350 *broteri* distribution and lineage divergence. Soil texture (i.e. sand content) and pH were
351 the most relevant variables. Whereas pH is known as a key predictor for the occurrence
352 of plant species, since it affects the availability of nutrients and phytotoxic metals
353 (Wagner *et al.*, 2017), soil texture mainly influences the water holding capacity and
354 therefore it is important in the adaptation to Mediterranean dry biomes (Saxton & Rawls,
355 2006; Padilla & Pugnaire, 2007).

356 Autopolyploidy, niche evolution and competitive interactions

357 We observed consistent environmental gradients fostering niche evolution in *D. broteri*
358 complex, and we identified two patterns of polyploid niche shifts within this series.
359 Whereas we found evidence of niche expansion in tetraploids (related to diploids), the
360 higher polyploids demonstrated a trend to occupy specialized niches in narrow and
361 stressful habitats (i.e. niche novelty *sensu* Marchant *et al.*, 2016). Diploid and tetraploid
362 niches were similar but the tetraploid cytotype showed a more widespread range
363 according to its wider niche breadth. Although a similarity test between these *D. broteri*
364 cytotypes was previously performed by Glennon *et al.* (2014) and gave congruent results,
365 it failed to find differences between niches and the overlap was remarkably higher (0.70
366 vs 0.53). Such differences could be due to the inclusion of topo-edaphic factors as well
367 as other more meaningful climatic variables for Mediterranean plants (e.g. *PETs*, climatic
368 moisture index or precipitation of warmest quarter; Detto *et al.*, 2006; Dubuis *et al.*,
369 2013).

370 However, the niche overlap pattern is clearly different when each 4x lineage was
371 considered independently. We found niche equivalence and the highest overlap in the
372 comparison 2x-4x_s but different, non-overlapping and divergent niches between 2x and
373 4x_e. The differences in genetic isolation and divergence of both tetraploid lineages from
374 diploids (Balao *et al.*, 2010) might help to explain this pattern. Nevertheless, diploids and
375 tetraploids do not actually coexist (absence of mixed-cytotype populations) and therefore
376 other ecological factors may have driven the geographic segregation. In this context, the
377 disjunct distribution of diploids (Balao *et al.*, 2009) might be due to competitive exclusion
378 in arid localities by southern tetraploids (4x_s), whose phenotypic changes (Balao *et al.*,
379 2011a) could help colonize competitive environments as suggested for the southern Spain
380 populations of *Brachypodium distachyon* tetraploids (Rey *et al.*, 2017). Another non-

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

389 The higher polyploids (6x and 12x cytotypes) have diverged towards
390 environmental margins, and occur in specialized and mostly non-overlapping niches with
391 regard to the other cytotypes. This niche divergence pattern is similar to that found in a
392 broad survey within the tribe Potentilleae of the Rosaceae family, which included more
393 than 100 species and six different ploidy levels (Brittingham *et al.*, 2018). In *D. broteri*,
394 6x and 12x niches were characterized by the most extreme conditions (high temperatures
395 and scarce water availability) of the Mediterranean climate. The hexaploid cytotype
396 inhabits a semi-arid Mediterranean area in SE Spain notable for the absence of
397 seasonality, with a low precipitation and high temperatures, and *PETs* limiting the wettest
398 periods (López-Bermúdez, 1990). Furthermore, the 6x niche was also characterized by
399 eroded, unfertile (i.e. low organic carbon content) and basic soils as expected in arid or
400 semi-arid habitats (Barea *et al.*, 2011). These features constrain plant growth and cause
401 functional specialization and diversity (Rundel *et al.*, 2016). Interestingly, hexaploids also
402 share a portion of the described environmental niche with eastern tetraploids (mostly soils
403 with high pH and harsh climatic conditions in the wettest quarter), which were probably
404 involved in their origin by hybridization with southern diploids (Balao *et al.*, 2010). The
405 origin of the dodecaploids is unclear but, based on the molecular and genome size data,
406 it seems that they have evolved largely independently of the other cytotypes (Balao *et al.*,
407 2009; Balao *et al.*, 2010). This 12x cytotype has acquired adaptations to allow it to survive
408 the harsh summer (with low precipitations and high temperatures) in the Doñana National
409 Park area (S Spain; Zunzunegui *et al.*, 2005), where it is currently distributed. In addition,
410 this area is characterized by sandy soils with high bulk density (i.e. paleodunes) which
411 inherently have a low available water content (McNabb *et al.*, 2001; Obia *et al.*, 2016).
412 These soil properties have major effects on plant growth (Maun, 1994; Place *et al.*, 2008;
413 Tracy *et al.*, 2013).

414 Phylogenetic niche conservatism and recurrent polyploid origins

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

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

447 2017), and where the apparent competitive superiority of the higher cytotypes may be
448 enhanced by their recurrent polyploid formation and the alleged unfilled niches of lower
449 ploidies.

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

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461 **Author contributions**

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

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740

741 **Figure legends**

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

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

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

763 **Fig. 4** Phenograms for the two main environmental axes constructed with data
764 corresponding to the *Dianthus broteri* population phylogeny ($n = 25$) from Balao *et al.*
765 (2010). The tables on the upper left corners summarize each model performed with AICc
766 values and their weights. Labels on the right margin indicate the names of the populations.
767 Asterisks mark the eastern tetraploid clade.

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771 **Supporting Information**

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

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

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

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

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

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

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

786 **Methods S1** Predictor layers can be downloaded from public databases. All R scripts for
787 niche comparison tests, habitat suitability models and PNC analyses are available at a

788 GitHub repository DOI: 10.5281/zenodo.2388457
789 (<https://github.com/fbalao/envdianthus/tree/v1.0>).

790 **Tables**

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

<i>D. broteri</i> lineages	<i>n</i>	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	-

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

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

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

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

<i>AWC</i>	Available soil water capacity	0.6	0.0	1.2	0.0	0.0	0.0	0.0
<i>BLDFIE</i>	Bulk density (fine earth) in kg / cubic-meter	0.4	0.0	0.2	0.0	0.0	0.1	0.0
<i>CECSOL</i>	Cation exchange capacity of soil in cmolc/kg	1.5	0.4	1.2	1.2	0.0	0.0	0.2
<i>ORCDRC</i>	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
<i>PHIHOX</i>	Soil pH x 10 in H ₂ O	2.9	2.2	16.2	6.5	0.0	0.3	2.2
<i>SNDPPT</i>	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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803 **Table 3** Ecological niche comparisons for *Dianthus broteri* using cytotyped and GBIF
 804 records. Columns correspond to the different tests performed in the two main PCA-env
 805 axes: overlapping, similarity (all the comparisons in both directions), equivalency and
 806 environmental divergence (derived from McCormack *et al.*, 2010)

<i>D. broteri</i> lineages			Niche similarity			Environmental divergence test	
<i>a</i>	<i>b</i>	Niche overlap (<i>D</i>)	<i>a</i> → <i>b</i>	<i>b</i> → <i>a</i>	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**

807 ns, not significantly different/equivalent

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

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

812 C, conservatism.

813 D, divergence.