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3 Do floral and niche shifts favour the establishment and persistence
4 of newly arisen polyploids? A case study in an Alpine primrose

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16 RUNNING TITLE: Roles of floral and niche shifts in polyploid establishment and persistence

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1 ABSTRACT

2 **Background and Aims** Polyploidization plays a key role in plant evolution. Despite the generally
3 accepted ‘minority-cytotype exclusion’ theory, the specific mechanisms leading to successful
4 establishment and persistence of new polyploids remain controversial. The majority of newly
5 formed polyploids do not become established, because they are less common, have fewer potential
6 mates, or may not be able to successfully compete with co-occurring progenitors at lower ploidy
7 levels. Changes in floral traits and ecological niches have been proposed as important mechanisms
8 to overcome this initial frequency-dependent disadvantage. We aim at determining whether
9 dodecaploids of the heterostylous *P. marginata* differ from their hexaploid progenitors in *P.*
10 *marginata* and *P. allionii* for selected floral traits and ecological preferences that might be involved
11 in establishment and persistence, providing a possible explanation for the origin of polyploidized
12 populations.

13 **Methods** We quantify and compare floral morphological traits and ecological niche preferences
14 among dodecaploids and their hexaploid progenitors in *P. marginata* and *P. allionii*, all restricted to
15 the southwestern Alps.

16 **Key Results** We detect differences in floral traits between dodecaploids and their closest relatives,
17 but such differences might be too weak to counter the strength of minority cytotype disadvantage
18 and are unlikely to enable the co-existence of different cytotypes. Furthermore, our results suggest
19 the preservation of full distyly and no transition to selfing in dodecaploids. Finally, dodecaploids
20 occur almost exclusively in environments that are predicted to be suitable also for their closest
21 hexaploid relatives.

22 **Conclusions** In light of our results, *P. marginata* dodecaploids have probably been able to establish
23 and persist by occupying geographical areas not yet filled by their closest relatives without
24 significant evolution in their climatic and pollination niches. Dispersal limitation and minority-
25 cytotype exclusion probably maintain their current range disjunct from those of its close relatives.

26

1 **Key words:** ecological niche, minority-cytotype exclusion, pollinator shift, polyploidy, *Primula*
2 *allionii* Loisel., *Primula marginata* Curtis.

3

4 INTRODUCTION

5 Polyploidy has long been recognized as an important mechanism of genome evolution and
6 as playing a key role in plant speciation (Grant, 1981), usually causing immediate reproductive
7 isolation between plants of the original ploidy and the newly arisen polyploids (e.g., triploid block
8 between diploids and tetraploids; Husband and Sabara, 2004). However, reproductive isolation is
9 not sufficient for polyploid speciation (Sobel *et al.*, 2010). In order to become established, newly
10 formed polyploids must increase their fitness in comparison to their progenitors either through
11 selfing or through increasing the probability of mating with individuals at the same ploidy level
12 (rather than with individuals of different ploidy) via shifting floral traits or blooming period.
13 Additionally, they can also increase their fitness through occupying an ecological niche and/or a
14 geographic space that differ from those of their relatives at lower ploidy, thereby decreasing
15 competition with them. Therefore, the evolutionary success of polyploids depends on a series of
16 complex reproductive, morphological, ecological, and geographic factors (Felber-Girard *et al.*,
17 1986; Lumaret *et al.*, 1987; Husband, 2000; Ramsey and Schemske, 2002).

18 Given the rare occurrence of unreduced gamete formation (Bretagnolle and Thompson,
19 1995; Ramsey and Schemske, 1998, 2002), which represents the first step in the most common
20 mode of polyploidization, newly formed polyploids usually occur at low frequency in sympatry
21 with their closest relatives, hence they may suffer a competitive disadvantage. Most neopolyploids
22 are thus expected to quickly disappear (Soltis *et al.*, 2010; Arrigo and Baker, 2012). The ‘minority-
23 cytotype exclusion’ principle (MCE; Levin, 1975) states that the proportion of the rarer cytotype
24 decreases rapidly as a result of frequency-dependent selection. Indeed, the rarer cytotype is at a
25 reproductive disadvantage, because there is a greater likelihood that its pollen will be transferred to

1 the more frequent cytotype, thus preventing the formation of fertile seeds. The reproductive
2 handicap in a given generation leads to a greater handicap in the next, rapidly eliminating the
3 minority cytotype from the population (Levin, 1975; Burton and Husband, 2000; Husband, 2000).
4 Because newly formed polyploids occur in sympatry with at least one progenitor, changes in plant
5 morphology, phenology, physiology and/or ecological preferences are deemed necessary to
6 overcome the initial frequency disadvantage that they inevitably experience and allow their
7 establishment and persistence (Levin, 2002). There might thus exist strong selective pressures for
8 neopolyploids to either differ in their floral traits (thus decreasing the frequency of mating with
9 their diploid ancestors), exploit new niches, or better cope with fluctuating environments, in order
10 to outcompete or avoid competition with their closest relatives (Petit *et al.*, 1999; Baack, 2004;
11 Leitch and Leitch, 2008; te Beest *et al.*, 2012).

12 Polyploidization is known to alter floral traits (Otto and Whitton, 2000; Robertson *et al.*,
13 2010; Balao *et al.*, 2011), often causing a shift in pollinator preferences (Muchhala and Potts, 2007;
14 Whittall and Hodges, 2007; Blüthgen *et al.*, 2008; Waterman *et al.*, 2011; Gómez *et al.*, 2014). For
15 example, polyploidization may trigger an increase in organ size, which might affect floral traits
16 involved in pollinator attraction (e.g., corolla-width; Balao *et al.*, 2011; Tunbridge *et al.*, 2011),
17 flower-pollinator interactions during nectar probing (e.g., corolla-tube length and width; Segraves
18 and Thompson, 1999; Balao *et al.*, 2011; Tunbridge *et al.*, 2011), or pollen transfer (e.g., positions
19 of male and female sexual organs in the floral tube; Keller *et al.*, 2012, 2014). Shifts of floral traits
20 between diploids and polyploids may play an important role in promoting assortative mating within
21 the same cytotype, possibly leading to preferential pollen transfer among minority-cytotype
22 individuals, despite their lower frequency in a population (Segraves and Thompson, 1999; Husband
23 and Sabara, 2004; Kennedy *et al.*, 2006).

24 Furthermore, polyploids frequently occupy different portions of environmental space and
25 may expand or shift their geographical ranges relative to the progenitors, sometimes in connection

1 with cycles of habitat and range fragmentation driven by climatic oscillations (Stebbins, 1984;
2 Guggisberg *et al.*, 2006, 2009; Spooner *et al.*, 2010; Fawcett and Van de Peer, 2010; te Beest *et al.*,
3 2012; Theodoridis *et al.*, 2013). The spatial segregation of cytotypes via differentiation of their
4 environmental niches is thought to be one of the primary mechanisms involved in alleviating MCE
5 (Fowler and Levin, 1984; Thompson and Lumaret, 1992), and it has been suggested as a
6 ‘prerequisite’ for polyploid speciation (Levin, 2003).

7 While evidence is growing that different mechanisms can lead to the establishment and
8 persistence of new polyploids, the consistency and generality of the proposed mechanisms are still
9 debated (Soltis *et al.*, 2010; Glennon *et al.*, 2014). Therefore, analyses of floral and ecological
10 differences between cytotypes at different ploidy levels within and between closely related species
11 are needed to better understand the mechanisms of polyploid evolution. Here, we focus on two
12 species of primroses, *Primula marginata* Curtis and *P. allionii* Loisel., which are restricted to the
13 south-western Alps and are closely related, being included in the monophyletic *Primula* sect.
14 *Auricula* (Mast *et al.*, 2001, 2006; Zhang *et al.*, 2004; Casazza *et al.*, 2012). This ancestrally
15 hexaploid section comprises species with genomes that are likely to be diploidized (Boucher *et al.*,
16 2016). Furthermore, allopatric speciation with little niche divergence, hence largely non-adaptive,
17 appears to have been the most common mode of speciation in this section (Boucher *et al.*, 2016).
18 All species in the section have dimorphic flowers with female (stigmas) and male (anthers) sexual
19 organs placed reciprocally in long-styled (L) and short-styled (S) plants (Richards, 2003), a system
20 designed to prevent self-fertilization and known as heterostyly (Webb and Lloyd, 1986;
21 Wedderburn and Richards, 1992). In *Primula*, polyploidisation is often associated with a shift from
22 self-incompatible heterostyles, characterized by pronounced anther-stigma separation within each
23 floral morph, to self-compatible homostyles, characterized by greatly reduced or no anther-stigma
24 separation in the single floral morph. The described shift should favor the establishment of the
25 newly formed polyploids (Wedderburn and Richards, 1992; Guggisberg *et al.*, 2006).

1 *Primula marginata*, described as heterostylous by Richards (2003), comprises populations at
2 either the hexaploid ($2n = 6x = 62, 66$; hereafter *Pm6x*) or dodecaploid ($2n = 12x = 120-128$;
3 hereafter *Pm12x*) levels. No populations with mixed *P. marginata* cytotypes (*Pm12x* and *Pm6x*)
4 have been identified (Casazza *et al.*, 2012), while two mixed populations of *Pm12x* and *P. allionii*
5 individuals (hereafter *Pa6x*) are known (Casazza *et al.*, 2013a). Hexaploid and dodecaploid
6 populations of *P. marginata* are geographically differentiated (Fig. 1), with *Pm6x* individuals
7 occurring in the western (*i.e.*, from the Cottian to the south-western Maritime Alps) and *Pm12x*
8 individuals in the eastern part of the species' range (*i.e.*, from the south-eastern Maritime and
9 Ligurian Alps to the Apennines; Casazza *et al.*, 2013b). Recently, *Pm12x* individuals have been
10 proposed to be allopolyploids originating from hybridization between *Pm6x* and *Pa6x* lineages
11 (Casazza *et al.*, 2012).

12 The present study is aimed at characterizing for the first time selected floral traits in both
13 floral morphs and climatic preferences that might explain the establishment and persistence of
14 polyploidized populations in heterostylous species, focusing on the Alpine *P. marginata*-*P. allionii*
15 system. More specifically, we are asking: 1) Do *Pm12x* individuals differ from *Pm6x* and *Pa6x*
16 individuals in floral traits that are involved in pollinator attraction and handling, potentially
17 promoting assortative mating between individuals of the same cytotype? 2) Are *Pm12x* individuals
18 characterized by decreases in floral display traits and anther-stigma distance that might indicate a
19 switch to self-fertilization? 3) Do *Pm12x* populations deviate from full distyly? 4) Do the ecological
20 preferences of *Pm12x* populations differ significantly from those of its progenitors and, if so, could
21 such differences explain geographic segregation among related lineages at different ploidy levels?
22 5) Do *Pm12x* populations have broader ecological tolerances that would allow them to occupy a
23 broader geographical range than those of their hexaploid relatives? The results of the study should
24 allow us to better understand the mechanisms of polyploid establishment and persistence.

25

26 MATERIAL AND METHODS

1 *Study system*

2 Individuals of *Pa6x* form dense evergreen cushions bearing single pink flowers with a white
3 eye. The species is the only known primrose characterized by petals with a bright surface, similarly
4 to other plants pollinated by bumblebees. Individuals of this species bloom from February to April
5 and are pollinated only by Hymenoptera (Minuto *et al.*, 2014). Individuals of *Pm6x* and *Pm12x*
6 form loose cushions with stems usually bearing from 5 to 10 flowers from lilac to pinkish with a
7 mealy eye-ring. Individuals of *Pm6x* and *Pm12x* do not show obvious morphological differences
8 and they bloom from March to June (Richards, 2003). While no study on pollinators of *Pm6x* and
9 *Pm12x* individuals has been published, according to our personal observations both taxa are
10 pollinated by Hymenoptera and Lepidoptera.

11

12 *Floral traits and sexual organ position*

13 We collected flowers from nine hexaploid and eight dodecaploid populations of *P.*
14 *marginata* and two populations of the hexaploid *P. allionii* (Supplementary Data Table S1; see also
15 Casazza *et al.*, 2012; 2013a). The number of analysed flowers was 121 (47 from the L-morph and
16 74 from the S-morph) for *Pm6x*, 164 (80 L-morph and 84 S-morph) for *Pm12x*, and 100 (48 L-
17 morph and 52 S-morph) for *Pa6x* (see Supplementary Data Table S1 and S2 for further details).

18 All flowers were longitudinally dissected and analysed under a Leica M205 C
19 stereomicroscope equipped with a Leica EC3 digital colour camera; selected traits were measured
20 using the LAS EZ imaging software (Leica). We focused on floral traits involved in pollinator
21 attraction and flower-pollinator interaction (see de Vos *et al.*, 2014). We analyzed two traits
22 implicated in pollinator attraction: flower number and size. Specifically, we measured the total
23 number of blooming flowers per scape during the whole flowering period (NF) (recorded only in
24 *Pm12x* and *Pm6x* individuals on 94 and 115 scapes respectively, because *Pa6x* individuals usually
25 bear a single flower per scape; Richards, 2003) and petal lobe length (LL) as a proxy for flower
26 size. We analyzed two traits implicated in flower-pollinator interaction (Fig. 2): corolla tube length

1 (TL) and corolla mouth diameter (MD). To assess the potential ability of flowers to self-pollinate,
2 we quantified the degree of anther-stigma separation (*i.e.*, herkogamy) for each flower as the
3 difference between the distance from the middle of anthers (anther position: AP) to the tip of the
4 stigma (stigma position: SP). To check for potential deviations from full distyly in dodecaploid
5 populations of *P. marginata*, we also calculated the sexual reciprocity index of Sánchez *et al.*
6 (2008) in the three target taxa at different ploidy levels. This index, which we label R , compares
7 stigma–stamen height differences for all potential crosses (*i.e.*, stamen position of each morph
8 versus stigma position of each complementary morph across a population), while accounting for the
9 standard deviation of these measurements. This index has the advantage of not being skewed by the
10 most frequent sex organ (stamens) and can be interpreted as a measure of the average population-
11 level deviation from perfect reciprocity ($R = 0$); distyly is usually characterized by $R < 0.05$. In the
12 few distylous species of *Primula* in which R was calculated it ranges from 0.007 to 0.020 (Keller *et al.*
13 *et al.*, 2012, 2014, 2016; Aronne *et al.*, 2014). We used an analysis of similarity (ANOSIM; Clarke,
14 1993) to test the degree and the significance of differences between floral traits of *Pm12x*
15 individuals and those of their relatives in all measured floral traits (excluding NF) of each morph.
16 ANOSIM is a non-parametric test that generates a statistic comparing dissimilarities between and
17 within groups and usually ranging from 0 to 1, with 0 indicating completely random grouping and
18 no difference between groups. Negative values may occur, indicating a greater dissimilarity among
19 individuals within than between groups. ANOSIM was preferred to parametric tests (e.g.,
20 MANOVA) because our data contained a few strong outliers that might have affected the
21 performance of the latter. Euclidean distances were used in all comparisons and the significance of
22 the statistic was calculated with 9,999 random permutations. In order to visualize the pattern of
23 morphological variation among cytotypes, a principal component analysis (PCA) was carried out.
24 To test whether the two morphs of *Pm12x* individuals differed significantly from the respective
25 morphs of their relatives in all measured floral traits, we used a Kruskal–Wallis test in the R
26 statistical software v 3.0.1 (R Development Core Team, 2012).

1

2 *Occurrence data and climatic variables*

3 Locality data were extracted from Favarger (1965), Kress (1969, 1989), and Casazza *et al.*
4 (2012) for hexaploid and dodecaploid populations of *Primula marginata* and from Casazza *et al.*
5 (2013a) for *P. allionii*. The final georeferenced dataset consisted of all populations of *P. marginata*
6 for which the ploidy level is known (19 for *Pm6x* and 17 for *Pm12x*) and all known populations of
7 *P. allionii* (54 for *Pa6x*).

8 Climatic variables related to temperature and precipitation are thought to be important for
9 physiological limitations and adaptation in alpine plants (Körner, 2003). We thus downloaded
10 nineteen bioclimatic variables from the WorldClim database (<http://www.worldclim.org>) at a 30-s
11 (*i.e.* about 1x1 km) spatial resolution (Hijmans *et al.*, 2005) for the south-western Alps. Because
12 (micro-)topographic features can strongly affect climate in alpine habitats (Körner, 2003) and
13 because the resolution of the environmental layer used in modelling may affect model prediction
14 (Guisan *et al.*, 2007; Randin *et al.*, 2009; Kirchheimer *et al.*, 2016), we employed a finer spatial
15 resolution. The coarse-scale (1-km resolution) climatic grids were statistically downscaled to 100-m
16 resolution, following the approach detailed in Zimmermann *et al.* (2007). To reduce multi-
17 collinearity between predictors and minimize model over-fitting, we measured pairwise Pearson's
18 correlation coefficients among values of all climatic predictors extracted by occurrences points and
19 retained a set of predictors with a high relative contribution to the species distribution models
20 (SDMs), but not highly correlated with each other ($r \leq |0.70|$; see recommendation from Elith *et al.*,
21 2006). The five variables used in further analyses were: Mean Diurnal Range of Temperature
22 (BIO2); Temperature Seasonality (BIO4); Mean Temperature of Wettest Quarter (BIO8), Mean
23 Temperature of Driest Quarter (BIO9), Precipitation Seasonality (BIO15); Precipitation of Warmest
24 Quarter (BIO 18); and Precipitation of Coldest Quarter (BIO19).

25

26 *Niche differentiation in environmental space*

1 Values of environmental variables were extracted for all localities of *Pm12x*, *Pm6x* and
2 *Pa6x* populations. We used a non-parametric Kruskal–Wallis test to assess differences between
3 *Pm12x* and their closest relatives along each climatic variable independently. Kernel density plots
4 were also used to visualize the distribution of each variable.

5 A detectable niche shift between two or more species may be either the result of niche
6 differentiation in environmental space (E-space) that translates into the occupation of different
7 geographic space (G-space) or a consequence of differences in the availability of the environment in
8 G-space with no niche differentiation in E-space (Soberón, 2007; Broennimann *et al.*, 2012;
9 Theodoridis *et al.*, 2013). To test for differentiation in E-space, a principal components analysis
10 (PCA)-based method recently developed by Broennimann *et al.* (2012) was used to quantify niche
11 breadth and niche overlap of *Pm12x*, *Pm6x* and *Pa6x* populations. Overlap was measured by
12 Schoener's *D* index, which ranges from 0 (no overlap) to 1 (full overlap; Schoener, 1970). All
13 occurrences were then pooled and randomly split into two datasets 100 times, maintaining the
14 number of occurrences as in the original datasets to compare the niche overlap statistic *D* with a
15 random distribution.

16 To test whether ecological niches of the taxa being compared are more similar than expected
17 at random from their geographical ranges, we use niche similarity tests (Warren *et al.*, 2010). Niche
18 similarity tests compare the set of environmental conditions occupied by different taxa via taking
19 into account the background environmental conditions that are available in the geographic area
20 occupied by each taxon. Briefly, the observed climatic niche overlap between two species was
21 compared to the overlap measured between one of the species' niche and the randomized niche of
22 the other species. This randomized niche was obtained by randomly sampling occurrence points in
23 the region where the species occurs (the 'background area'). We repeated this randomization
24 procedure 100 times. We created different background areas following two different approaches. A
25 first approach used the outputs of the species distribution models (SDMs) for *Pm12x*, *Pm6x*, and
26 *Pa6x* separately (see below). Probability of presences from SDMs were converted into binary

1 predictions using a base-line threshold based on the point in the Receiver Operating Curve (ROC)
2 where the sum of sensitivity and specificity is maximized (Manel *et al.*, 2001; Liu *et al.*, 2005), and
3 background maps of predicted presences were generated. In addition, we also used a geographic
4 background obtained from combining the SDMs of all three taxa (common background). A second
5 approach used 10-, 20- and 30-km buffer zones around the occurrence points of each species
6 (Warren *et al.*, 2010).

7 Niche breadth in E-space of each species was assessed by randomly sampling 100 pixels in
8 the niches of *Pm12x*, *Pm6x* and *Pa6x* populations (*i.e.*, pixels were sampled according to the density
9 of the species occurrences), extracting their scores along the two PCA axes, and calculating the
10 standard deviation of the scores along each axis (Broennimann *et al.*, 2012). This procedure was
11 repeated 1000 times and the distribution of the breadth values was compared using boxplots for
12 each PCA axis.

13 All analyses of niche overlap, niche similarity and niche breadth were performed in R using
14 the set of functions provided in Broennimann *et al.* 2012 and implemented in the “ecospat” package
15 (Broennimann *et al.*, 2015).

16

17 *Niche differentiation in geographical space*

18 Occurrence data and bioclimatic variables were used for the construction of SDMs for each
19 taxon with Maxent 3.3.3e (Phillips *et al.*, 2006). Maxent is a very flexible modeling algorithm
20 widely used because of its high predictive performance (Elith *et al.*, 2006) even with low sample
21 sizes (down to as few as 10 points; Hernandez *et al.*, 2006; Pearson *et al.*, 2007). We used 70% of
22 the occurrence records for each taxon to calibrate the model and 30% to test it, as recommended
23 (Phillips *et al.*, 2006). All other parameters were set to default. Model performance was assessed
24 using the area under the receiver operating characteristic curve (AUC; Hanley and McNeil, 1982).

25 The niches of different taxa were also compared in G-space using a niche similarity test
26 implemented in ENMtools (Warren *et al.*, 2010). This test compares the observed overlap (again,

1 measured using Schoener's D) between niches of *Pm12x* populations and their relatives to a
2 distribution of simulated overlap values generated by comparing the SDM of one species to an
3 SDM created from random points drawn from the geographical background area of the other
4 species (Warren *et al.*, 2008). Significant results suggest that the ecological niches of sister species
5 are either more different or more similar than expected, and for this reason this test is treated as a
6 two-tailed test. The software ENMtools was used to perform niche similarity tests in G-space using
7 the same backgrounds as previously presented for E-space. Even if this approach is common, the
8 results of niche differentiation tests in G-space should be handled with caution, because measured
9 differences between niches could represent differences in the environmental characteristics of the
10 study area rather than real differences between species (Broennimann *et al.*, 2012).

11 Niche breadth in G-space was estimated by applying the inverse concentration metric of
12 Levins (1968) as implemented in ENMTools to the resulting sets of suitability scores, standardized
13 so that minimum possible niche breadth within this space is 0 (indicating that only one grid cell in
14 the geographical space has a non-zero suitability) and maximum niche breadth is 1 (where all grid
15 cells are equally suitable).

16

17 RESULTS

18 *Floral traits and sexual organ positions*

19 Multivariate analysis of floral traits using ANOSIM (*Pm12x* vs *Pm6x*: $R = 0.29$, $p < 0.01$;
20 *Pm12x* vs *Pa6x*: $R = 0.30$, $p < 0.01$) and PCA (Supplementary Data Fig. S1) suggested that *Pm12x*
21 individuals are weakly but significantly different from their closest relatives. Considering all traits
22 separately, individuals of *Pm12x* did not differ significantly from their closest relatives in number
23 of flowers per scape for both L- and S-morphs (Fig. 3A; Supplementary Data Table S3). Petal lobes
24 of *Pm12x* flowers were significantly longer than those of *Pm6x* and *Pa6x* for L-morphs, and than
25 those of *Pm6x*, but not *Pa6x*, for S-morphs (Fig. 3B; Supplementary Data Table S3), with average
26 petal lobe length ranging from 9.85 mm for *Pm12x* to 8.85 for *Pm6x* and 8.64 for *Pa6x* (L-morph)

1 and from 8.93 mm for *Pm12x* to 8.24 for *Pm6x* and 8.50 for *Pa6x* (S-morph). The corolla tubes of
2 *Pm12x* individuals were significantly longer and more herkogamous than those of their closest
3 relatives for both floral morphs (Fig. 3C, E; Supplementary Data Table S3), while corolla mouth
4 diameter of *Pm12x* individuals was intermediate between those of *Pa6x* and *Pm6x* for both morphs
5 (Fig. 3D; Supplementary Data Table S3). Values of sexual reciprocity index were always lower
6 than 0.05, indicating distyly, and did not differ significantly among *Pm12x* (0.0424), *Pa6x* (0.0434)
7 and *Pm6x* (0.0433). To summarize, *Pm12x* individuals differed only weakly from their closest
8 relatives in pollinator-attraction traits, but differed more markedly in traits involved in flower-
9 pollinator interaction and were characterized by a significantly higher degree of herkogamy.

11 *Niche differentiation in environmental space*

12 When comparing the climatic preferences of *Pm12x* and *Pm6x*, the values of both Mean
13 Diurnal Range of Temperature (BIO2) and Precipitation of Warmest Quarter (BIO18) differed
14 significantly, while only the values of BIO2 were significantly different between *Pm12x* and *Pa6x*
15 (Table 1). In particular, kernel density plots (Supplementary Data Fig. S2) showed that the niche of
16 *Pm12x* populations was characterized by lower values of Mean Diurnal Range (BIO2) and by a
17 wider range of values for Precipitation of the Warmest Quarter (BIO18) than those of both its
18 closest relatives. In general, *Pm12x* populations seemed to occur under slightly more oceanic
19 conditions than their closest relatives.

20 The PCA (Fig. 4A, B) identified two components that collectively explained 66.85% of the
21 total climatic variation between the cytotypes (PC1 = 43.1%; PC2 = 23.8%) and 68.1% of the total
22 variation between *Pm12x* and *Pa6x* populations (PC1 = 41.5%; PC2 = 26.7%). Three variables
23 (Mean Temperature of Wettest Quarter-BIO8, Mean Temperature of Driest Quarter-BIO9 and
24 BIO18) showed almost equal contributions to both axes, two variables (BIO2 and Precipitation
25 Seasonality-BIO15) were more strongly associated with PC1, while Temperature Seasonality
26 (BIO4) and Precipitation of Coldest Quarter (BIO19) were more strongly associated with PC2. The

1 niche of *Pm12x* largely overlapped with the niches of its closest relatives in E-space (Fig. 4C, D).
2 The niche of *Pm6x* was slightly wider than that of *Pm12x* and was characterised by a wider diurnal
3 and seasonal range of temperature (BIO2 and BIO4). On the contrary, the niche of *Pa6x* was
4 narrower than that of *Pm12x* populations (Fig. 4D). These results were in accordance with
5 univariate analyses obtained with the Kruskal–Wallis test. Niche breadth of *Pm12x* populations
6 along PC1 was equal to that of *Pm6x* populations, while *Pa6x* populations had a weakly narrower
7 niche breadth than *Pm12x* populations (Fig. 5A). Along PC2, the niche breadth of *Pm12x*
8 populations was narrower than that of *Pm6x*, but wider than that of *Pa6x* populations (Fig. 5B).
9 According to the classification scheme provided by Rödder and Engler (2011), average niche
10 overlap in E-space was very limited between *Pm12x* and *Pa6x* populations ($D = 0.117$ in Table 2)
11 and moderate or low between *Pm12x* and *Pm6x* populations ($D = 0.534$ in Table 2).

12

13 *Niche differentiation in geographical space*

14 AUC values of models produced by Maxent and interpreted using the classification of
15 Araújo *et al.* (2005) indicated excellent model performance (cross-validated AUC values of 0.972,
16 0.944 and 0.995 in *Pm12x*, *Pm6x* and *Pa6x* populations respectively). Niche-breadth analyses based
17 on the inverse concentration metric revealed that *Pm12x* populations have roughly the same niche
18 width as *Pm6x* populations (0.708 vs. 0.675), but a larger niche than *Pa6x* (0.708 vs. 0.177).
19 Schoener's D was higher for the pair *Pm12x* vs. *Pm6x* populations (0.797 in Table 2) and lower for
20 the pair *Pm12x* vs. *Pa6x* populations (0.451 in Table 2).

21

22 *Niche similarity tests in E- and G-space*

23 Consistently with the observed overlap between *Pm12x* and its closest relatives, tests of
24 niche similarity in E-space suggested that the ecological niches are more similar than expected
25 given their environmental backgrounds (Table 2). Results of background similarity tests in G-space
26 were partially congruent with results in E-space, showing that the niches of *Pm12x* and *Pm6x* are

1 more similar than expected by chance (Table 2). Results of background similarity tests between
2 *Pm12x* and *Pa6x* populations showed that the niche of *Pm12x* was less similar to the niche of *Pa6x*
3 than expected by chance, while the niche of *Pa6x* was more similar to the niche of *Pm12x* (Table 2).
4 To summarize, the niche of *Pm12x* is usually significantly more similar to the niche of *Pm6x* and it
5 is significantly different from the niche of *Pa6x* in G-space only.

6

7 DISCUSSION

8 *Do dodecaploids of P. marginata differ from their progenitors in floral traits?*

9 In general, autopolyploidy is expected to generate phenotypes that are extreme or novel
10 relative to parental lines (Levin, 1983), whereas allopolyploidy is expected to generate phenotypes
11 that are either intermediate between the parents (Thompson, 1991, Johnston *et al.*, 2001; Schranz
12 and Osborn, 2004), similar to one or the other parent (Abbot and Lowe, 2004), or a mosaic of
13 parental traits (Ramsey and Schemske, 2002; Schranz and Osborn, 2004; Murren and Pigliucci,
14 2005). In our system, both the ANOSIM analysis and the PCA suggest that, even if some
15 differences occur in single traits, *Pm12x* individuals do not markedly differ from their closest
16 relatives when considering all floral traits together (Supplementary Data Fig. S1). In particular, the
17 floral traits of *Pm12x* individuals significantly exceed the mean values of their closest relatives in
18 three (*i.e.*, petal lobe length, corolla tube length, stigma-anther distance) out of five cases, while the
19 remaining two traits are equal or intermediate between those of their closest relatives (*i.e.*, number
20 of flowers per scape and corolla mouth diameter, respectively; Fig. 3). These results are counter to
21 the current lack of taxonomic distinction between dodecaploids and hexaploids of *P. marginata*.

22 The comparisons of floral traits between the dodecaploids of *P. marginata* and their two
23 hexaploid relatives showed that *Pm12x* individuals have slightly larger petal lobes in three out of
24 four comparisons (Fig. 3B), but do not differ in the number of flowers per scape (Fig. 3A). Are such
25 small differences in floral size likely to affect pollinator attraction? It has been proposed that

1 changes in floral traits induced by polyploidy may promote a decrease in the frequency of inter-
2 ploidy pollination, thus allowing for the establishment of newly formed polyploids (Husband and
3 Sabara, 2004; Kennedy *et al.*, 2006; Godsoe *et al.*, 2013). A few studies have provided evidence
4 supporting the above prediction (Kennedy *et al.*, 2006; Husband and Schemske, 2000). However,
5 traits related to pollinator attraction are not universally larger in polyploids than in diploids and
6 differences in floral size may be important for polyploid establishment only under certain
7 conditions, i.e., when pollinators are choosy, scarce or unpredictable (Vamosi *et al.*, 2007).
8 Additionally, the importance of floral display in insect attraction is affected by phenology and
9 vegetation context (Sletvold *et al.*, 2013). In our case, competition for pollinators is low during the
10 partially overlapping flowering periods of the three studied primroses (Richards, 2003), because
11 few other species flower simultaneously in the same plant communities (Minuto *et al.*, 2014). As a
12 result, small differences in visual display between taxa may be relatively unimportant for early-
13 blooming taxa like the primroses investigated here.

14 In contrast with pollinator attraction, we detected greater differences between dodecaploids
15 of *P. marginata* and their progenitors for traits related to flower-pollinator interaction. Increment in
16 length and reduction in mouth width of the floral tube have been observed to reduce the number of
17 species of pollinator in flowers pollinated by bumblebees (Suzuki *et al.*, 2007) and hawkmoths
18 (Moré *et al.*, 2007). Flowers of *Pm12x* have corolla tubes that are longer, but corolla mouth
19 diameters that are intermediate to those of the progenitors (Fig. 3C, D). The dimensions of floral
20 tubes suggest that *Pm12x* flowers may be pollinated by a smaller than or the same range of
21 pollinators as *Pa6x* and *Pm6x*. In fact, the wider and shorter tubes of *Pa6x* individuals allow them to
22 be pollinated by a large number of pollinators irrespective of proboscis length (Minuto *et al.*, 2014).
23 The longer tubes of *Pm12x* plants may hinder the advantage of having tubes that are wider than
24 those of *Pm6x* individuals, allowing *Pm12x* flowers to be pollinated by the same or a subset of the
25 pollinators that visit *Pm6x* flowers (Fig. 3C-D). Considered together, our results suggest that the
26 differences between *Pm12x* individuals and their progenitors in floral traits that are known to affect

1 pollinator attraction and flower-pollinator interaction may not promote preferential mating among
2 *Pm12x* individuals through a shift in their pollinator preferences. In conclusion, while we have
3 detected differences in floral traits between *Pm12x* individuals and their closest relatives, these
4 differences might be too weak to counter the strength of minority-cytotype disadvantage and are
5 unlikely to enable the co-existence of different cytotypes. Additional field studies will be required
6 to explicitly test whether any differences in pollinator assemblages and fidelity exist between
7 *Pm12x* individuals and their closest relatives.

8 Polyploidization may be associated with a shift to self-fertilization (Barringer, 2007). While
9 polyploidization is often associated with increases in organ size, as discussed above, selfing renders
10 investment in pollinator attraction unnecessary, hence is often associated with smaller flowers
11 (Vamosi et al., 2007; Barringer and Geber, 2008). In *Primula*, polyploidization is usually
12 concomitant with a shift from distyly to homostyly and from self-incompatibility to self-
13 compatibility, concurrently with a marked reduction of anther-stigma distance (Richards, 2003;
14 Guggisberg et al., 2006; de Vos et al., 2014). In fact, the degree of herkogamy correlates negatively
15 with the degree of self-fertility (de Vos et al. 2012) and it may be considered a reliable proxy of
16 selfing rate. We detected an increase herkogamy in *Pm12x* individuals and virtually no differences
17 in levels of distyly between dodecaploid populations of *P. marginata* and their progenitors (Fig 3B,
18 E). Taken together, these results suggest preservation of full distyly and no transition to selfing in
19 *Pm12x* populations. Similarly, distyly is also preserved in *P. clusiana*, the only other dodecaploid
20 taxon of *P.* section *Auricula* (Zhang and Kadereit, 2004). The high herkogamy and the conservation
21 of heterostyly in *Pm12x* individuals corroborates the idea that polyploidization itself does not
22 necessarily trigger the switch from heterostyly to homostyly (Shore et al., 2006; Naiki, 2012).
23 Furthermore, the lacks of transition to selfing is in line with the increase of petal-lobe length in
24 *Pm12x* individuals, suggesting that flower size in polyploids may related to importance of
25 pollinators for plant reproduction (Vamosi et al., 2007).

1 *Do dodecaploids of P. marginata differ from their progenitors in ecological preferences?*

2 According to theories on polyploid speciation, spatial segregation of new polyploids via
3 differentiation of their environmental niches may allow them to escape MCE and become
4 established (Levin, 1975; Fowler and Levin, 1984; Thompson and Lumaret, 1992). However, the
5 role of niche shift in polyploid establishment and persistence continues to be the object of active
6 debate. Divergence of habitat preferences between related diploid and polyploid taxa was detected
7 in several studies (Parisod *et al.*, 2010; Glennon *et al.*, 2012; Manzaneda *et al.*, 2012; McIntyre,
8 2012; Kolář *et al.*, 2013, Sonnleitner *et al.*, 2016), including in *Primula* sect. *Aleuritia* (Theodoridis
9 *et al.*, 2013). Conversely, other studies, including reciprocal transplantation experiments, reported
10 no niche differentiation between polyploids and their related diploids (Baack and Stanton, 2005;
11 Godsoe *et al.*, 2013; Glennon *et al.*, 2014; López-Alvarez *et al.*, 2015). Therefore, other
12 mechanisms, including limited seed and pollen dispersal (Baack, 2005) and the capability of
13 polyploids to colonize recently deglaciated areas (Stebbins, 1984; Brochmann *et al.*, 2004; Godsoe
14 *et al.*, 2013; Theodoridis *et al.*, 2013), have been invoked to explain the establishment of
15 polyploids.

16 In line with these latter studies, our results show that the niche occupied by *Pm12x*
17 populations does not significantly differ from those of their closest relatives (Table 2; Fig. 3), even
18 though, as suggested by the moderate niche overlap values, the dodecaploids differ in their optimal
19 niche position (indicated by areas with more intense colour in Fig. 4, Table 1), preferring slightly
20 more oceanic conditions than their hexaploid relatives (Table 1; Figs S1 and S2). As suggested by
21 Glennon *et al.* (2014), this difference in optimal niche positions without global niche shift may be
22 the result of limited dispersal and competition between cytotypes for space. This suggests that
23 *Pm12x* populations may have taken advantage of the geographical space not yet filled by their
24 parents where more oceanic conditions exist without shifting their climatic niche. This
25 interpretation is congruent with the nearly allopatric range of *Pm12x* populations (Fig. 1) and the
26 previously proposed conclusion that allopatric speciation without ecological divergence is the main

1 mode of speciation in *P. sect Auricula* (Boucher *et al.*, 2016). The lack of niche shift supports the
2 idea that, for some species, mechanisms other than niche shift (e.g., limited dispersal capabilities or
3 biogeographic history) may be responsible for the success of polyploid establishment and
4 persistence.

5 Finally, theory suggests that newly formed allopolyploids should have broader ecological
6 tolerances than their progenitors through an increase in heterozygosity (Stebbins, 1971; Otto and
7 Whitton, 2000; Levin, 2002). However, evidence for the presumed broader ecological tolerances of
8 polyploids remains controversial and support for the opposite pattern is accumulating (Stebbins and
9 Dawe, 1987; Petit and Thompson, 1999; Martin and Husband, 2009; Glennon *et al.*, 2014,
10 Theodoridis *et al.*, 2013, Sonnleitner *et al.*, 2016). In the current study we found mixed evidence for
11 the two alternatives above, for the climatic niche of *Pm12x* was significantly wider than that of
12 *Pa6x*, but narrower than that of *Pm6x* (Fig. 5).

13 These differences in climatic niche breadth closely match differences in range size among
14 the three taxa: *Pa6x* is a narrow endemic restricted to two small areas of the Maritime Alps, *Pm12x*
15 has an intermediate range extending from the south-eastern Maritime and Ligurian Alps to the
16 Apennines, and *Pm6x* has the widest range of the three taxa, since it occurs from the Cottian to the
17 south-western Maritime Alps (Casazza *et al.*, 2013, see also Fig. 1). A similar relationship between
18 niche breadth and range size has been observed in other species, but the direction of causality
19 remains uncertain (Slatyer *et al.*, 2013). On the one hand, having a wider niche allows a species to
20 occupy a larger geographical range than species with a narrower niche. On the other hand, species
21 with a small geographic range are usually exposed to a narrower range of climatic conditions than
22 species with a larger range, resulting in a narrower realized niche (Sobéron 2007; Dullinger *et al.*,
23 2012). Moreover, other factors, including MCE, dispersal limitation, and lineage age might affect
24 the relationship between niche breadth and range size (Peterson *et al.*, 2011; Glennon *et al.*, 2014;
25 Theodoridis *et al.*, 2013). In our study group, the narrowest realized niche of *Pa6x* populations may
26 be the result of postglacial dispersal limitation (Casazza *et al.*, 2013a; Minuto *et al.*, 2014), as

1 observed in other Alpine endemics (Essl *et al.*, 2011). The narrower realized niche of *Pm12x*
2 compared to *Pm6x* populations may be due to the younger age of the allopolyploids. Thus, it is
3 likely that the differences in realized niche breadth detected among the studied taxa are largely due
4 to dispersal limitation, rather than to differences in fundamental niche breadth.

5

6 CONCLUSIONS

7 In this study, we detected minor differences in floral traits related to pollinator attraction,
8 and stronger differences in traits related to pollinator handling between *Pm12x* individuals and their
9 closest relatives. Although this remains to be properly assessed experimentally, such differences do
10 not seem to be sufficiently large to have promoted a consequential decrease in interploidy mating.
11 Furthermore, we did not find any evidence for a shift towards homostyly and selfing, but rather an
12 increase of herkogamy and corolla tube length in *Pm12x* individuals. Altogether, these results
13 suggest that differences in floral traits were likely insufficient for *Pm12x* individuals to establish
14 and persist by avoiding minority-cytotype disadvantage. This conclusion is also supported by the
15 observation that very few mixed populations of *Pm12x* and *Pa6x* and no mixed populations of
16 *Pm12x* and *Pm6x* are known to exist. In addition, we detected no clear shift of climatic niche or
17 broader ecological tolerances in *Pm12x* populations *versus* their closest relatives. These results,
18 combined with the nearly allopatric distributions of *Pm12x* and related taxa, suggest that a mainly
19 non-adaptive scenario is the most likely explanation for the persistence of *Pm12x*, as proposed for
20 speciation in the entire section *Auricula* (Boucher *et al.*, 2016). Our current results, combined with
21 evidence from previous molecular phylogenetic analyses (Casazza *et al.*, 2012), suggest that *Pm12x*
22 individuals originated from interspecific hybridization in a glacial refugium shared between *Pm6x*
23 and *Pa6x* individuals, and later persisted by shifting their range in geographical areas not yet filled
24 by their closest relatives without significant evolution of their climatic niche. Dispersal limitations
25 in the rugged south-western Alps and MCE probably maintain the current range of *Pm12x* largely
26 allopatric from those of its progenitors.

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Table 1. Results of Kruskal-Wallis statistical tests of differentiation for each climatic variable. The asterisks indicate a significant difference. *Pm6x* = *P. marginata* hexaploids; *Pm12x* = *P. marginata* dodecaploids; *Pa6x* = *P. allionii*.

Climatic variable	K-W	K-W
	<i>Pm6x</i> vs <i>Pm12x</i>	<i>Pa6x</i> vs <i>Pm12x</i>
BIO2 - Mean Diurnal Range	7.278 **	7.037 **
BIO4 - Temperature Seasonality	0.308 ^{ns}	1.148 ^{ns}
BIO8 - Mean Temperature of Wettest Quarter	0.603 ^{ns}	0.744 ^{ns}
BIO9 - Mean Temperature of Driest Quarter	0.145 ^{ns}	0.445 ^{ns}
BIO15 - Precipitation Seasonality	0.798 ^{ns}	1.614 ^{ns}
BIO18 - Precipitation of Warmest Quarter	4.784 *	0.291 ^{ns}
BIO19 - Precipitation of Coldest Quarter	0.845 ^{ns}	2.099 ^{ns}

** $P \leq 0.01$; * $P \leq 0.05$; ^{ns} $P \geq 0.05$.

Table 2. Results of niche similarity tests in environmental and geographical spaces among dodecaploids of *P. marginata* and their progenitors. Backgrounds are defined by each taxon's ecological niche model set to a baseline threshold that maximizes the sum of sensitivity and specificity of the test data (SDM), by the combination of SDM of each taxa (SDM CB), and by applying 10, 20 and 30-km buffer zones around the occurrence points of each species. Significant results are indicated by 'less' for significant divergence or 'more' for significant similarity between test and comparison cytotypes. *Pm6x* = *P. marginata* hexaploids; *Pm12x* = *P. marginata* dodecaploids; *Pa6x* = *P. allionii*.

	Test	Comparison	Niche overlap <i>D</i>	Background				
				SDM CB	SDM	10 Km buffer	20 Km buffer	30 Km buffer
Environmental space	<i>Pm12x</i>	<i>Pm6x</i>	0.269	more*	more*	more*	more*	more*
	<i>Pm6x</i>	<i>Pm12x</i>		more*	more*	more*	more*	more*
	<i>Pm12x</i>	<i>Pa6x</i>	0.193	more*	ns;	more*	more*	more*
	<i>Pa6x</i>	<i>Pm12x</i>		more*	more*	more*	more*	more*
Geographical space	<i>Pm12x</i>	<i>Pm6x</i>	0.797	more**	more**	more**	more**	more**
	<i>Pm6x</i>	<i>Pm12x</i>		more**	ns	more**	more**	more**
	<i>Pm12x</i>	<i>Pa6x</i>	0.451	less**	less**	less**	less**	less**
	<i>Pa6x</i>	<i>Pm12x</i>		ns	more*	ns	more**	more**

** $P \leq 0.01$; * $P \leq 0.05$ ns $P \geq 0.05$.

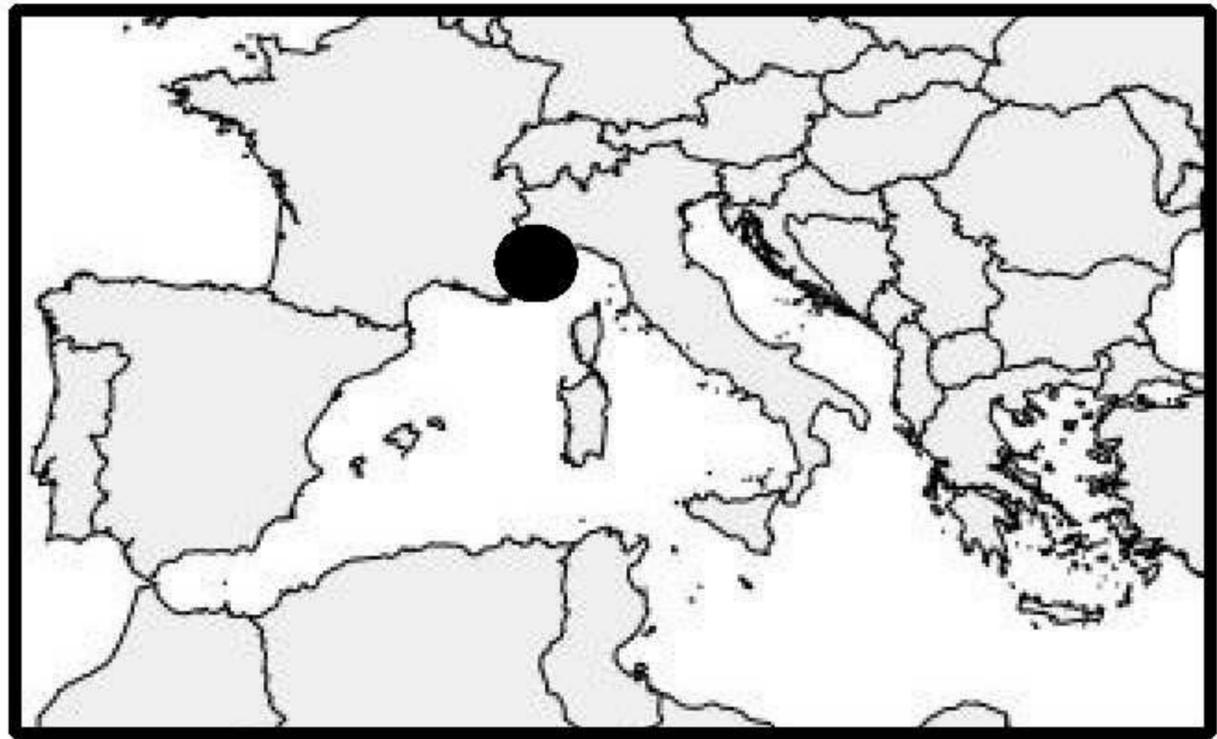
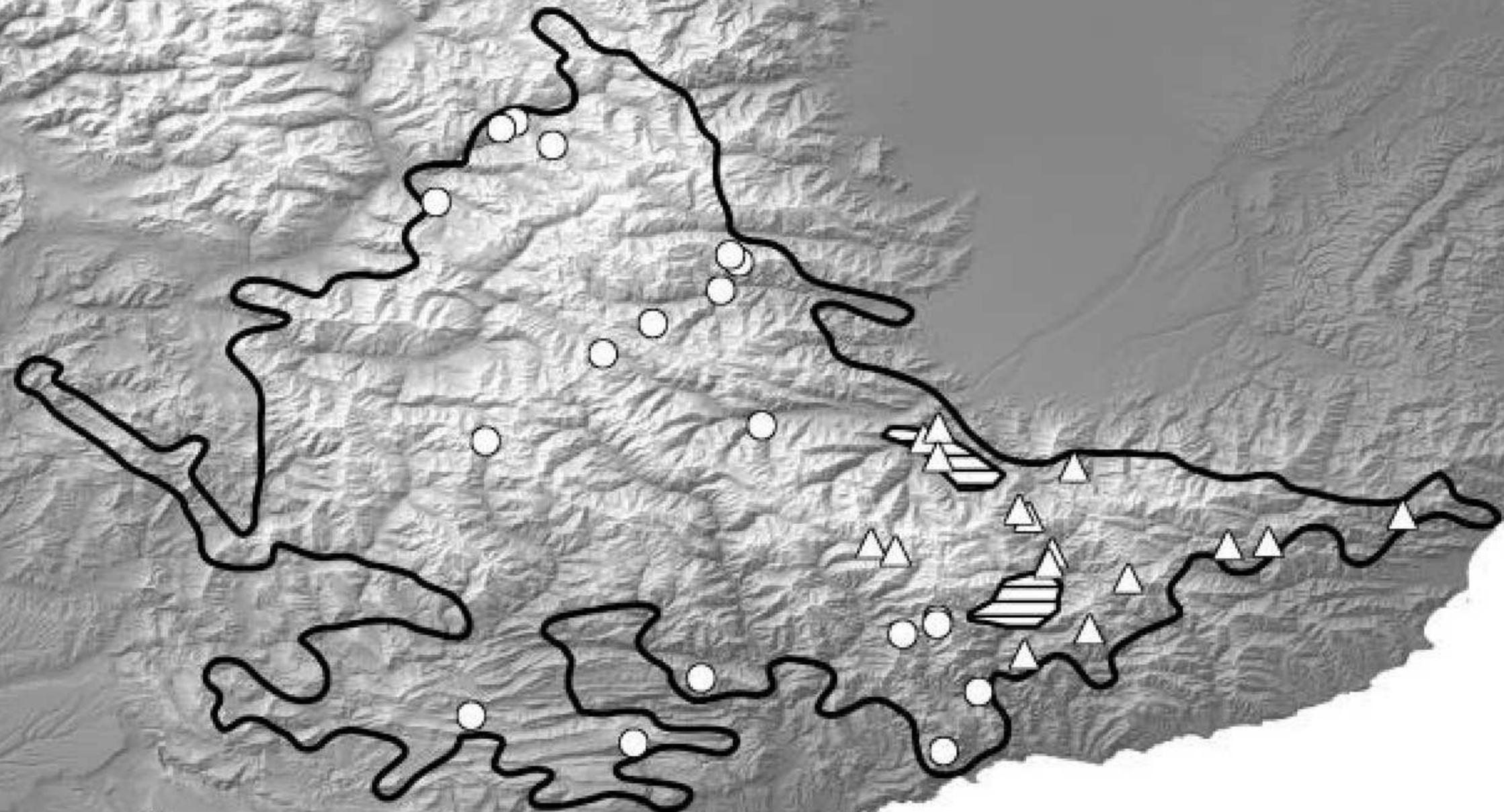
Figure 1. Distributional ranges of *P. marginata* (continuous black line) and *P. allionii* (white-striped areas). Cytotype distribution of *P. marginata* according to Kress (1969) and Casazza *et al* (2012): white circles = *P. marginata* hexaploids; white triangles = *P. marginata* dodecaploids.

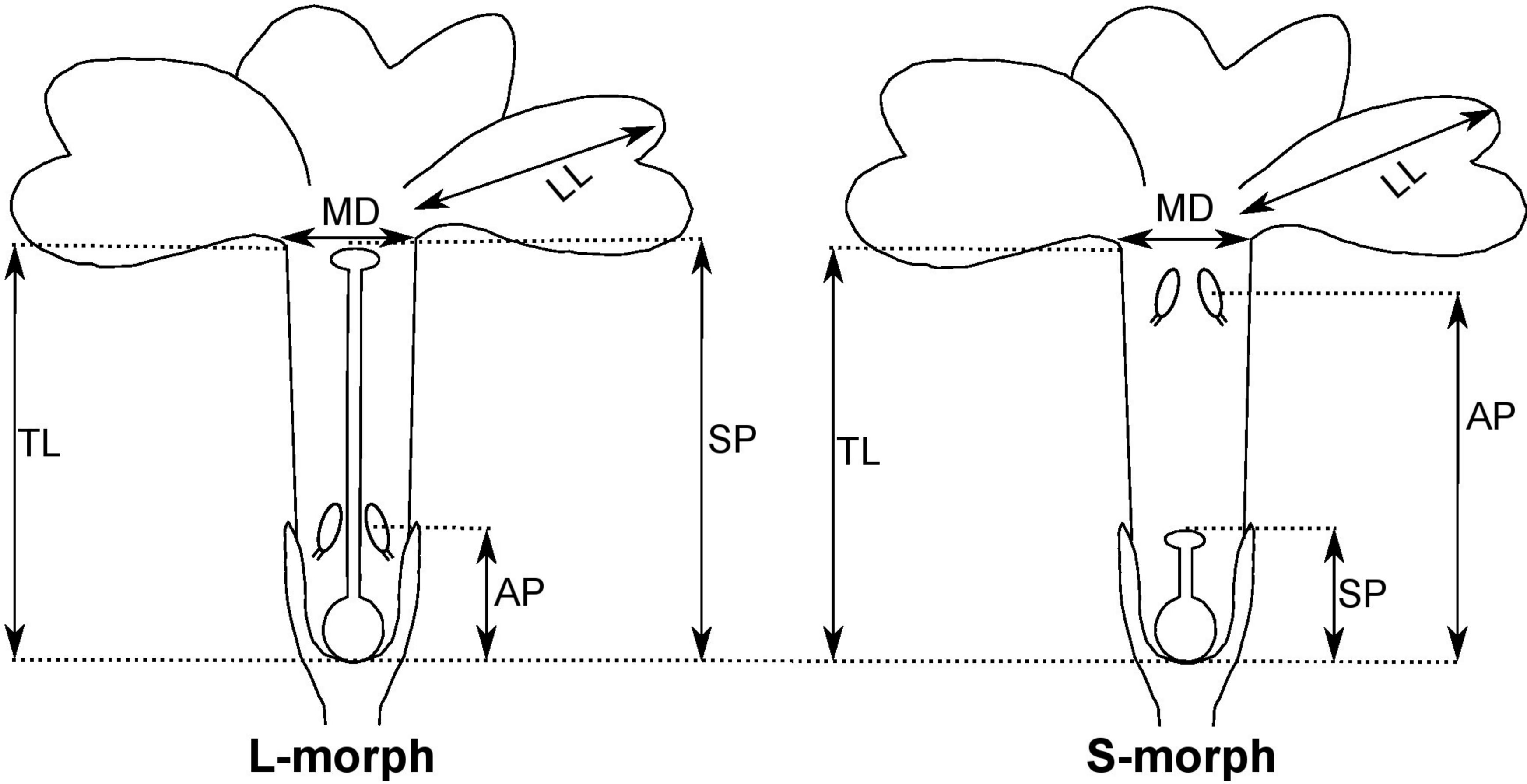
Figure 2. Floral traits measured in individuals of *P. marginata* (hexaploids and dodecaploids) and *P. allionii* (hexaploid species). Diagrams of long-styled (L) and short-styled (S) morphs. Sexual organ traits: AP, anther position; SP, stigma position. Corolla traits: TL, corolla tube length; MD, corolla mouth diameter; LL, petal limb length.

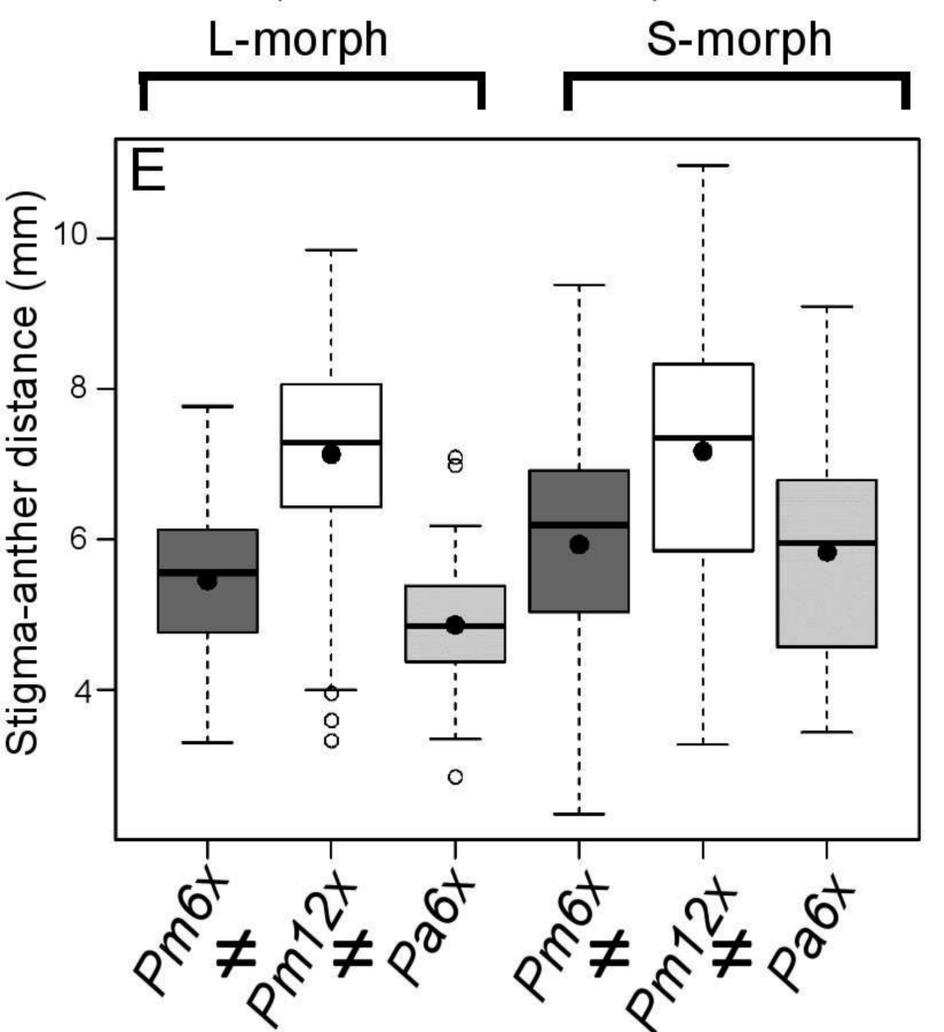
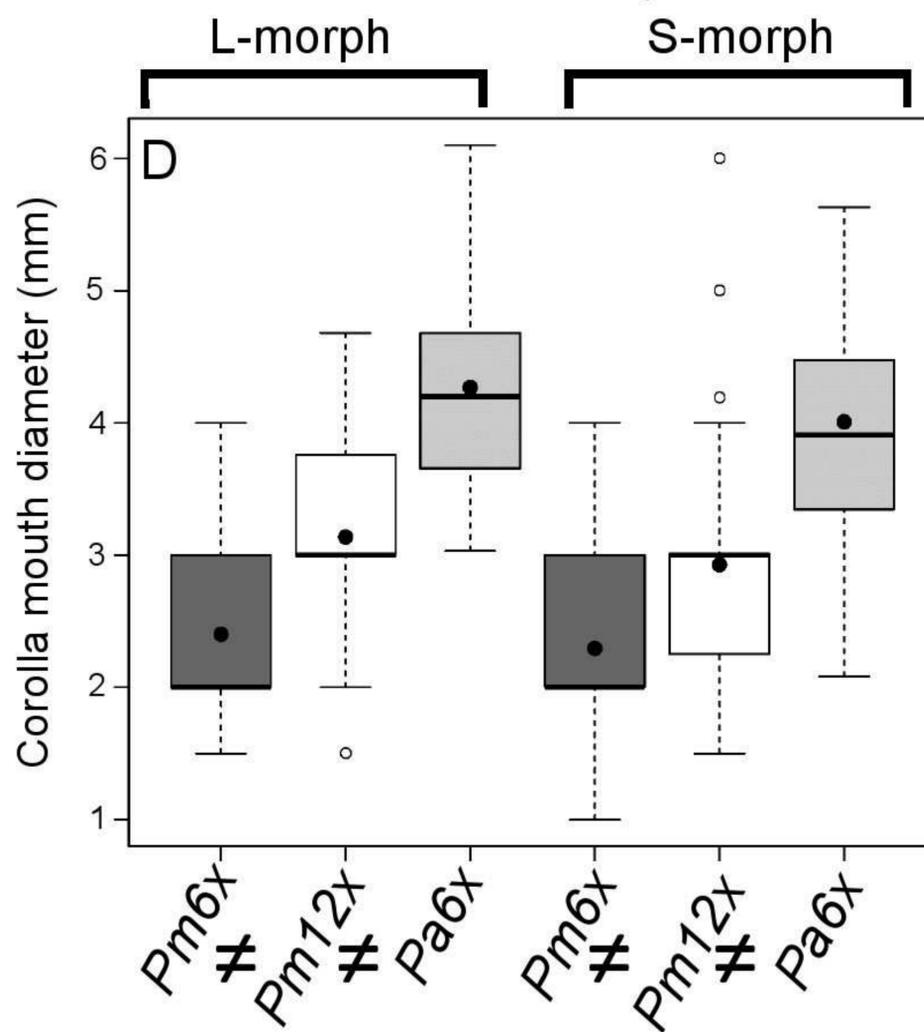
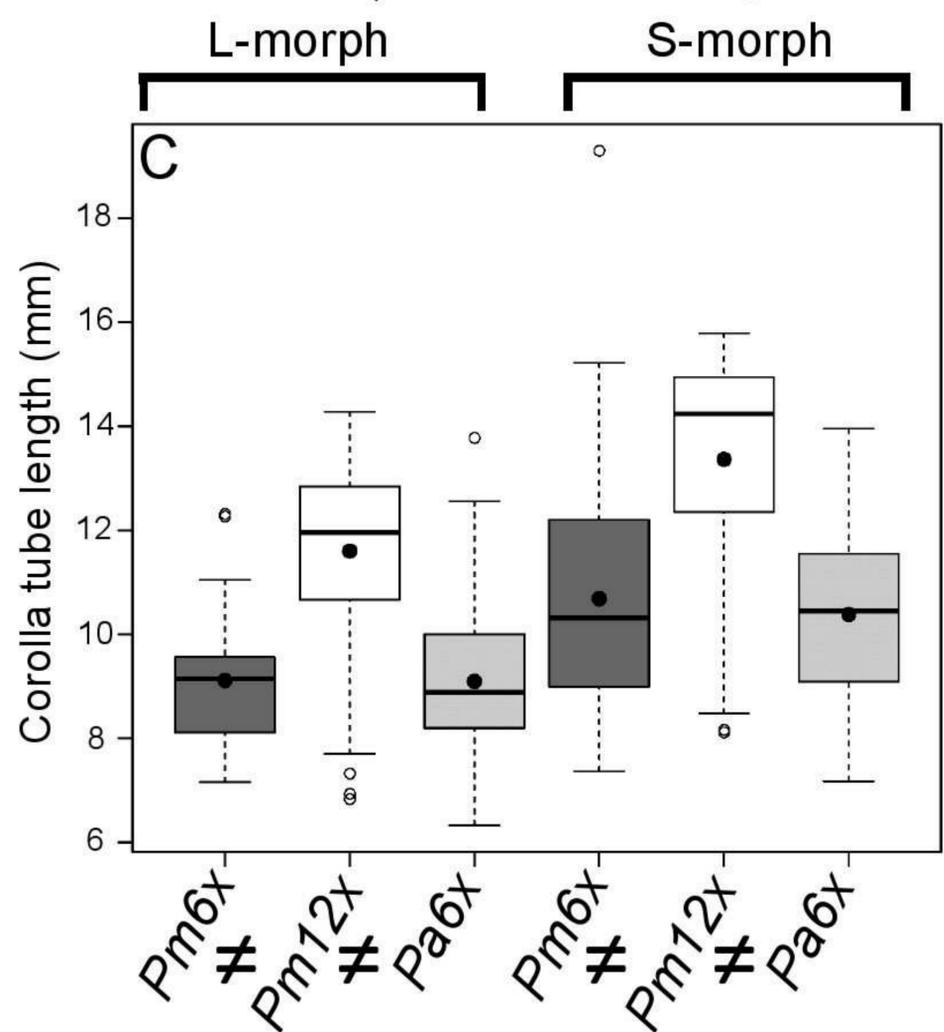
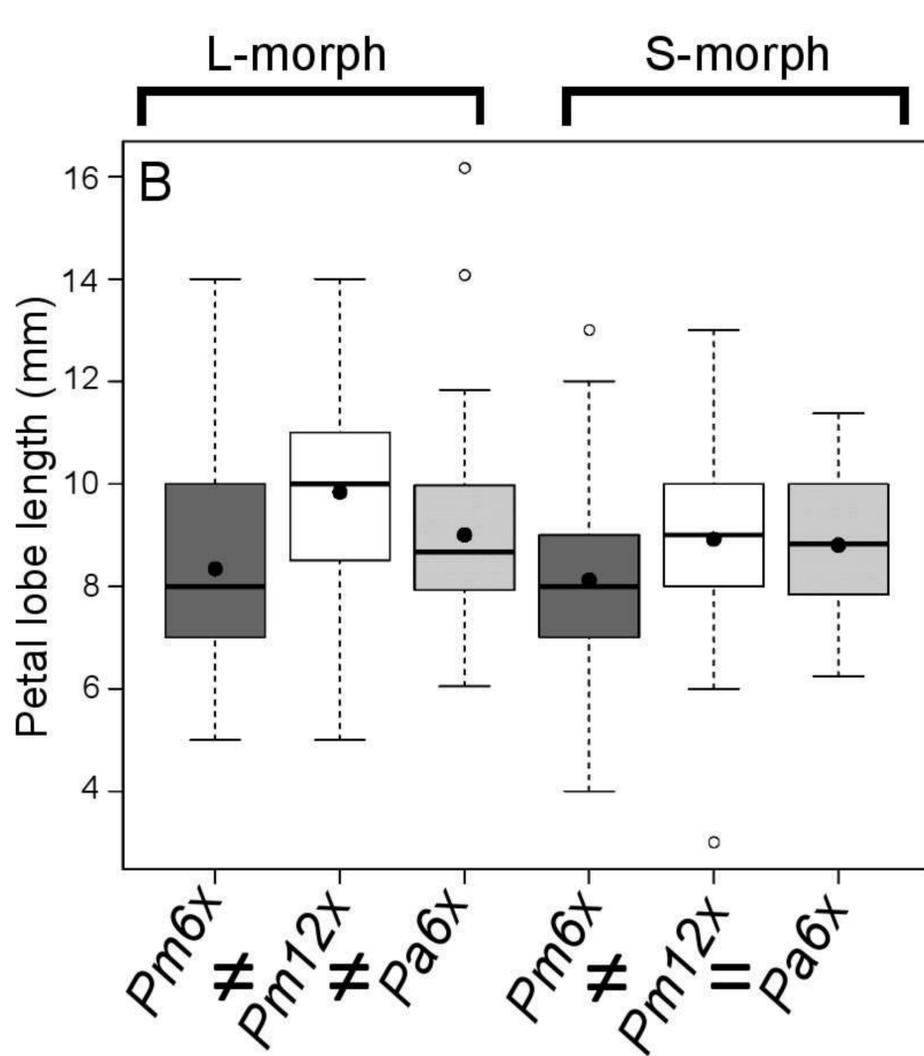
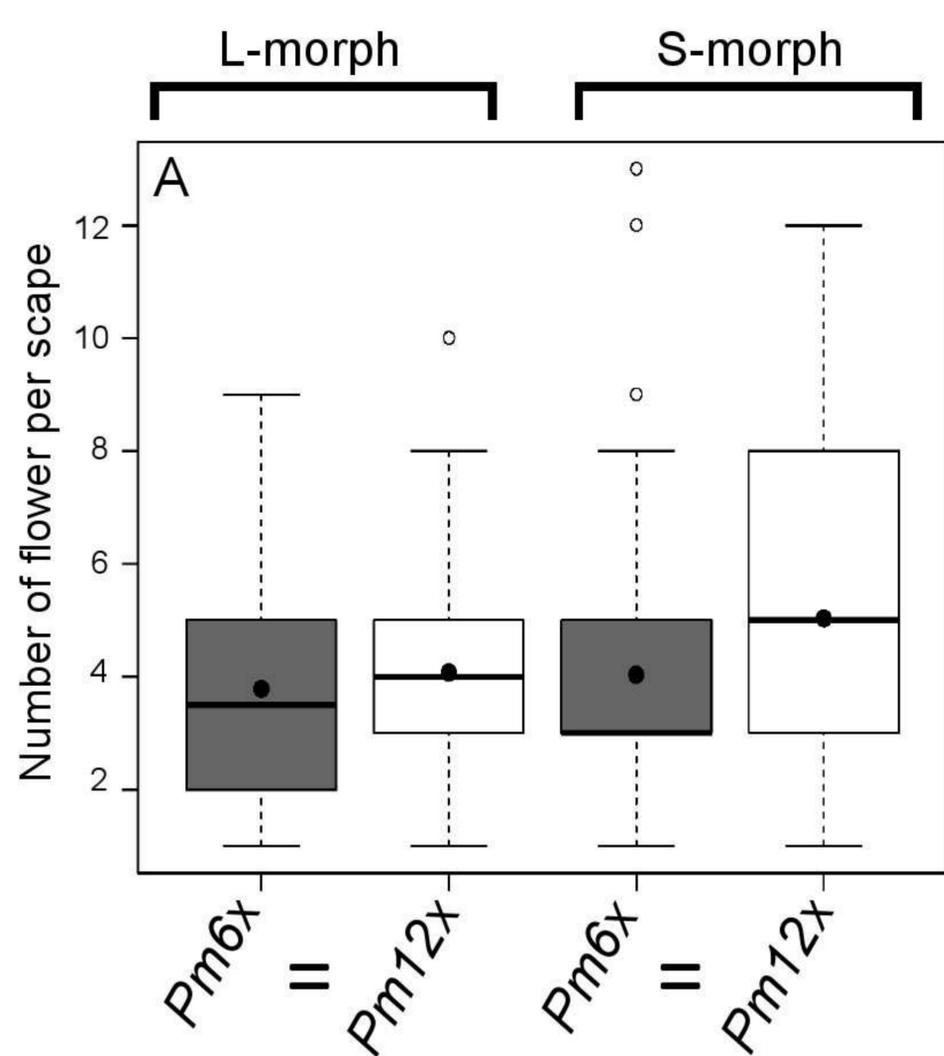
Figure 3. Boxplots of measurements of number of flowers per scape (A), petal limb length (B), corolla tube length (C), corolla mouth diameter (D) and stigma-anther distance (E). Data are reported for long-styled (L-morph) and short-styled (S-morph) morphs separately. *Pm6x* = *P. marginata* hexaploids; *Pm12x* = *P. marginata* dodecaploids; *Pa6x* = *P. allionii*. Results of Kruskal-Wallis tests for statistical differences are reported: a lack of significant difference ($P \geq 0.05$) is indicated by equality sign (=), while significant differences ($P < 0.05$) are indicated by inequality signs (\neq).

Figure 4. Niches of the dodecaploids of *P. marginata* (*Pm12x*), hexaploids of *P. marginata* (*Pm6x*) and *P. allionii* (*Pa6x*) in climatic space. Panels (A) and (B) show the contribution of climatic variables on the two PCA axes and the percentage of variation explained by these axes in *Pm12x* vs. *Pm6x* (A) and *Pm12x* vs. *Pa6x* (B), respectively. Panels (C) and (D) represent the niche of two pairs of taxa, *Pm12x* (blue) vs. *Pm6x* (red) and *Pm12x* (blue) vs. *Pa6x* (green), respectively. The solid and dashed lines represent 100 and 50% of the entire available environmental space (background), respectively. Colour shadings illustrate the density of the occurrences of *Pm6x*, *Pm12x* and *Pa6x* in each climatic cell. Notice the general overlap between niches but also the differences in mean niche position (areas with more intense colour), which result in moderate overlap measures.

Figure 5. Boxplots showing the distribution of niche breadth values of dodecaploids and their progenitors along the two PCA axes. Niche breadth was assessed by randomly sampling 100 pixels in the niche of each taxa, extracting their scores along the two PCA axes, and calculating the standard deviation of the scores along the two PCA axes. This procedure was repeated 1000 times. *Pm6x*=*P. marginata* hexaploids; *Pm12x* = *P. marginata* dodecaploids; *Pa6x* = *P. allionii*.







Kruskal-Wallis statistical test:

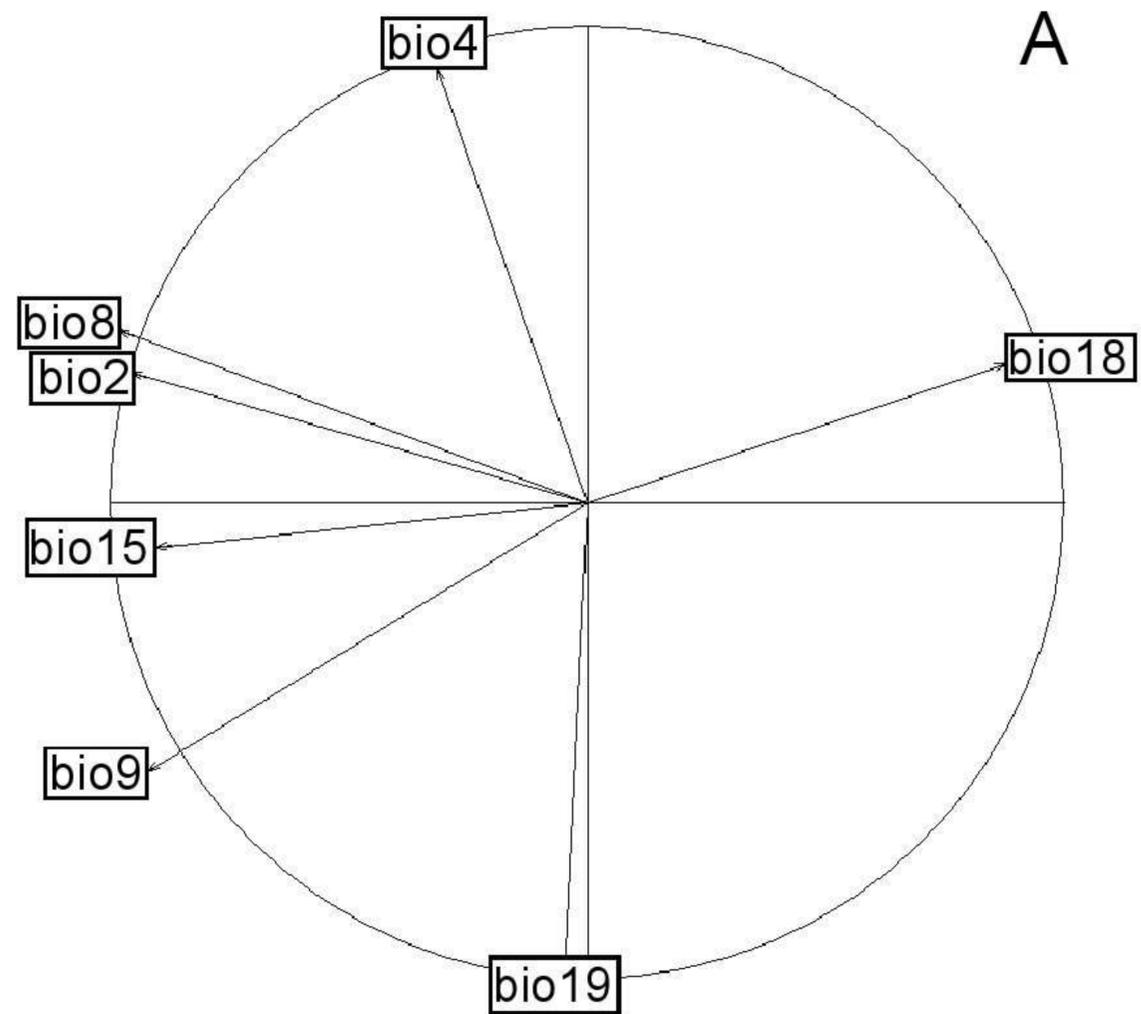
= $P \geq 0.05$; $\neq P < 0.05$

Pm12x: *P. marginata* dodecaploids

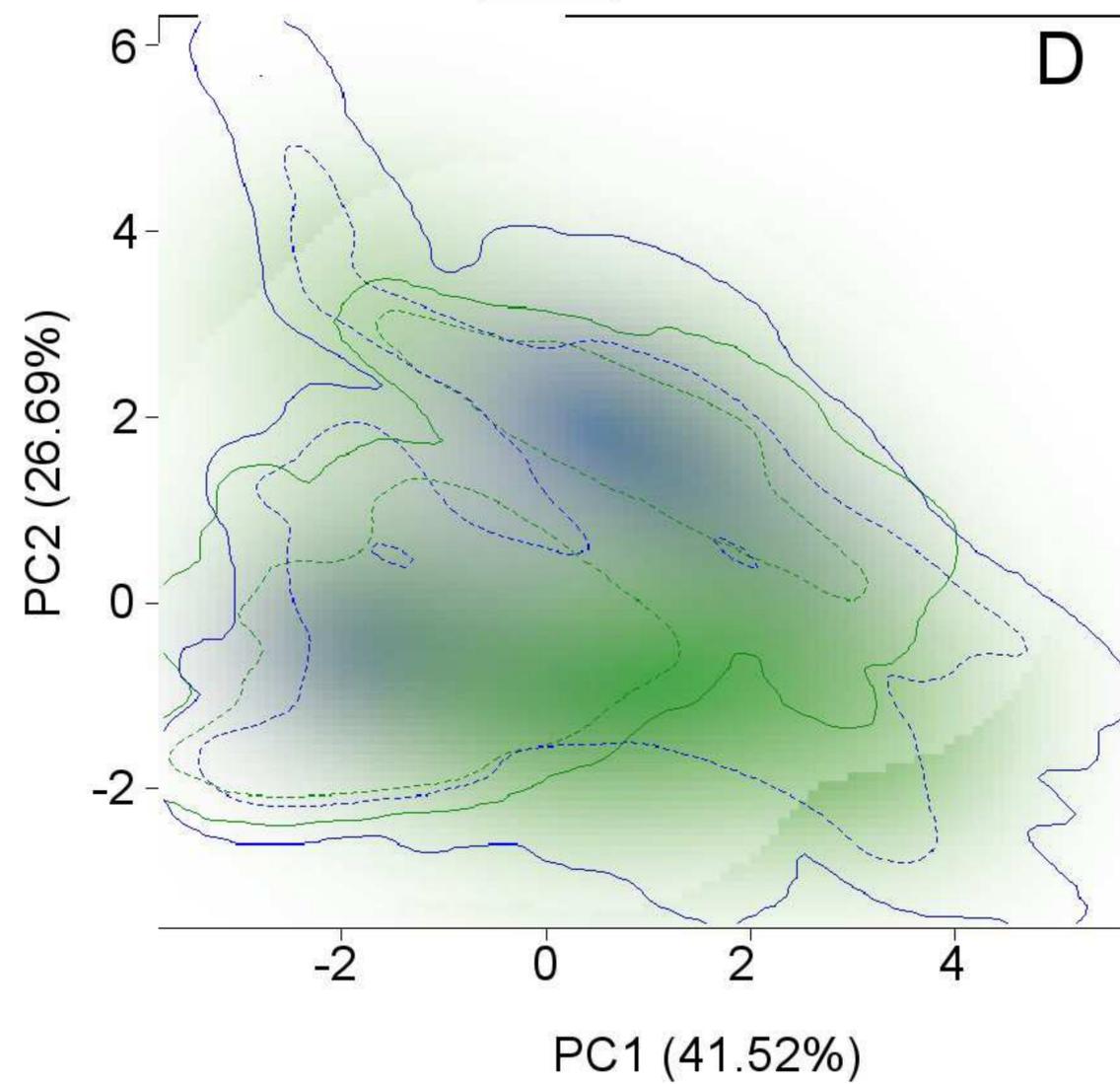
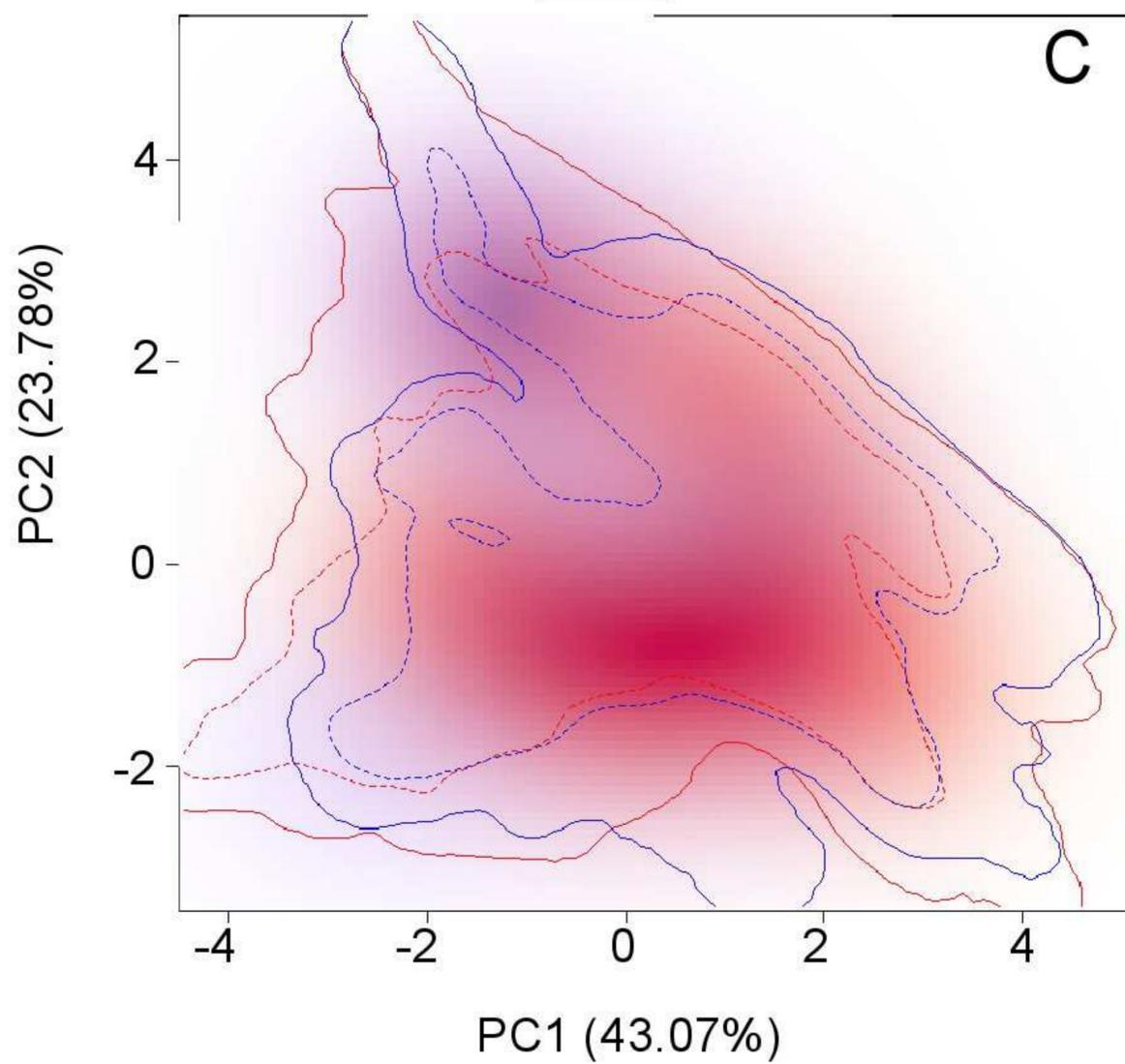
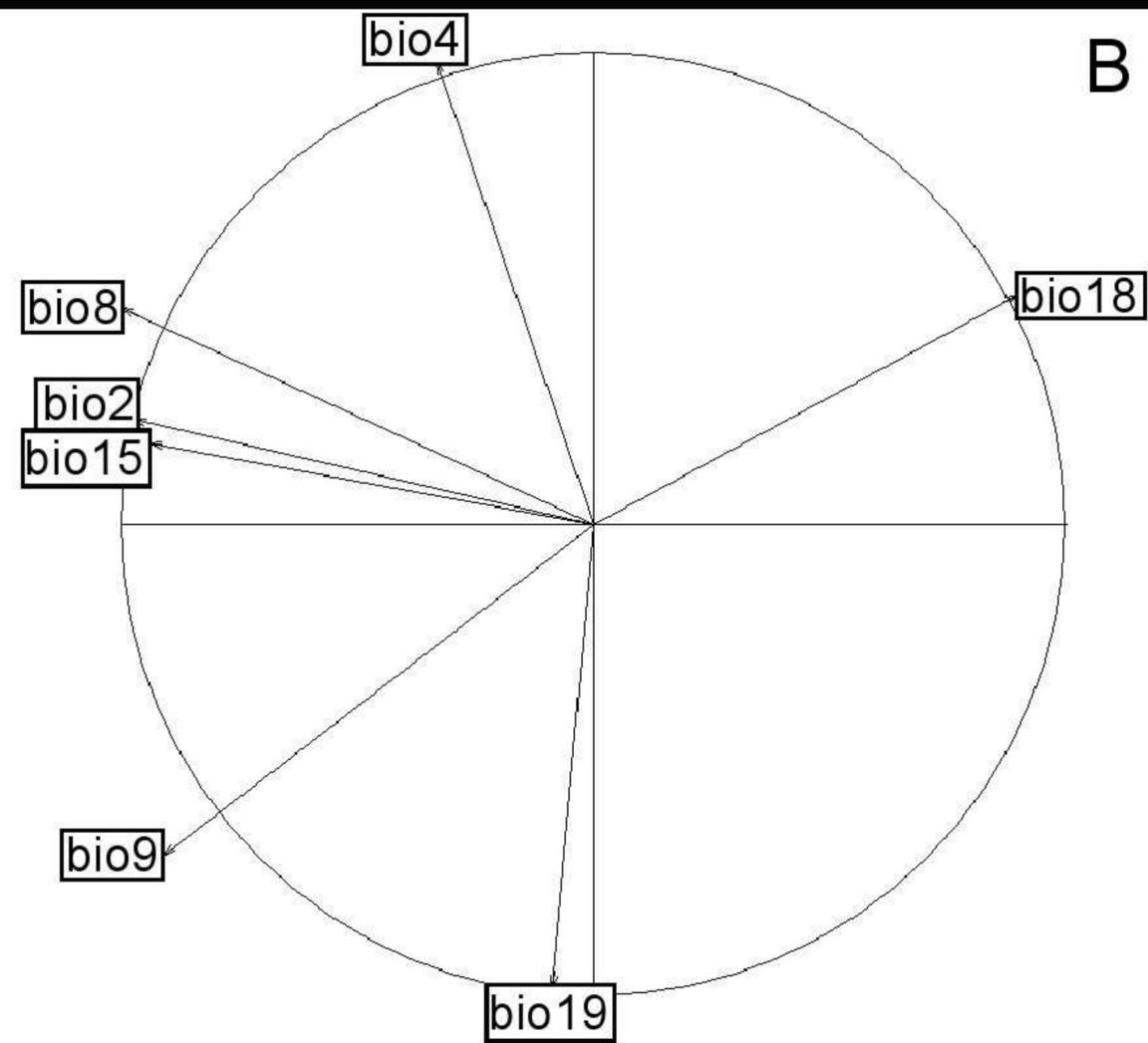
Pm6x: *P. marginata* hexaploids

Pa6x: *P. allionii* hexaploids

Pm12x vs Pm6x

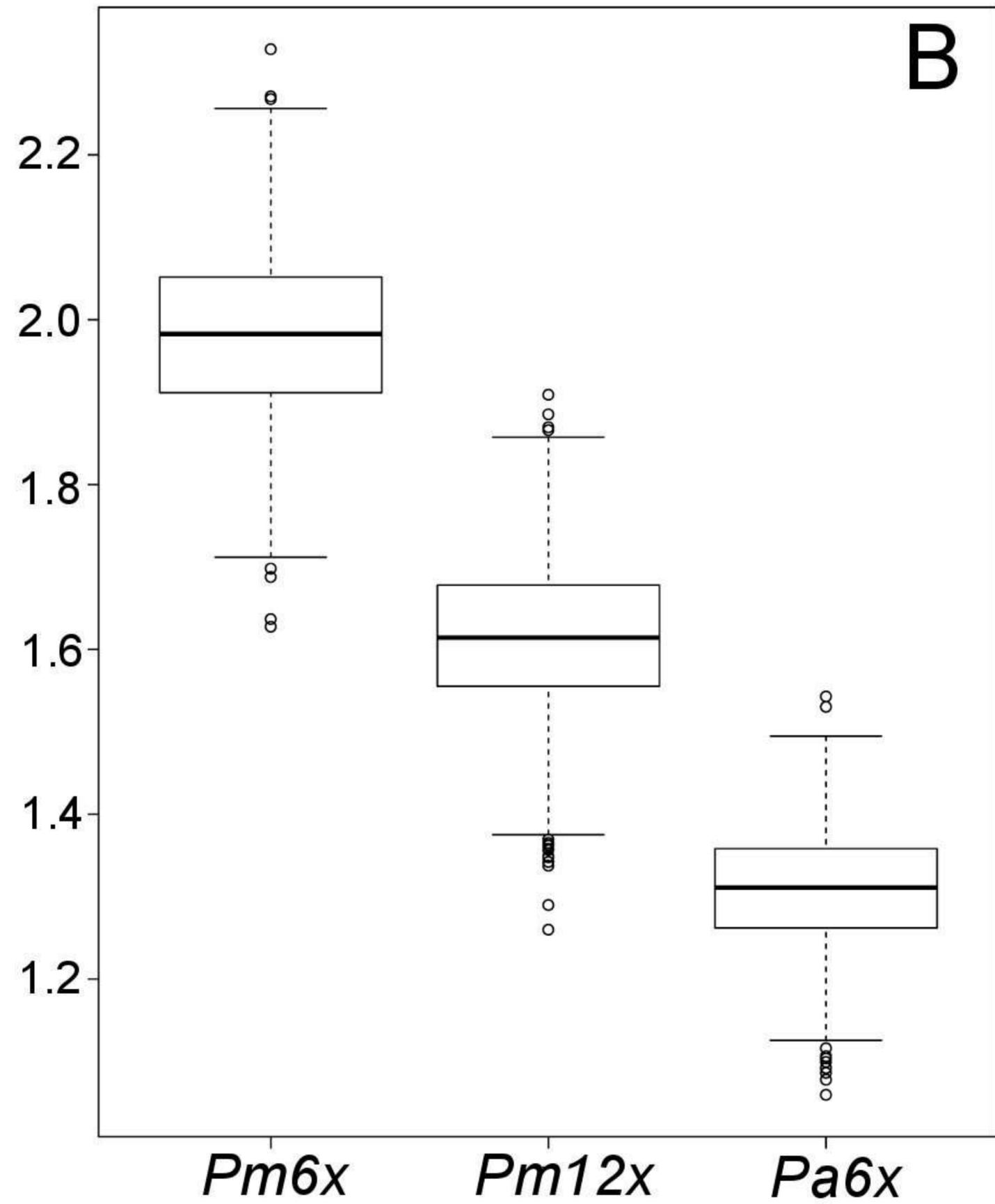
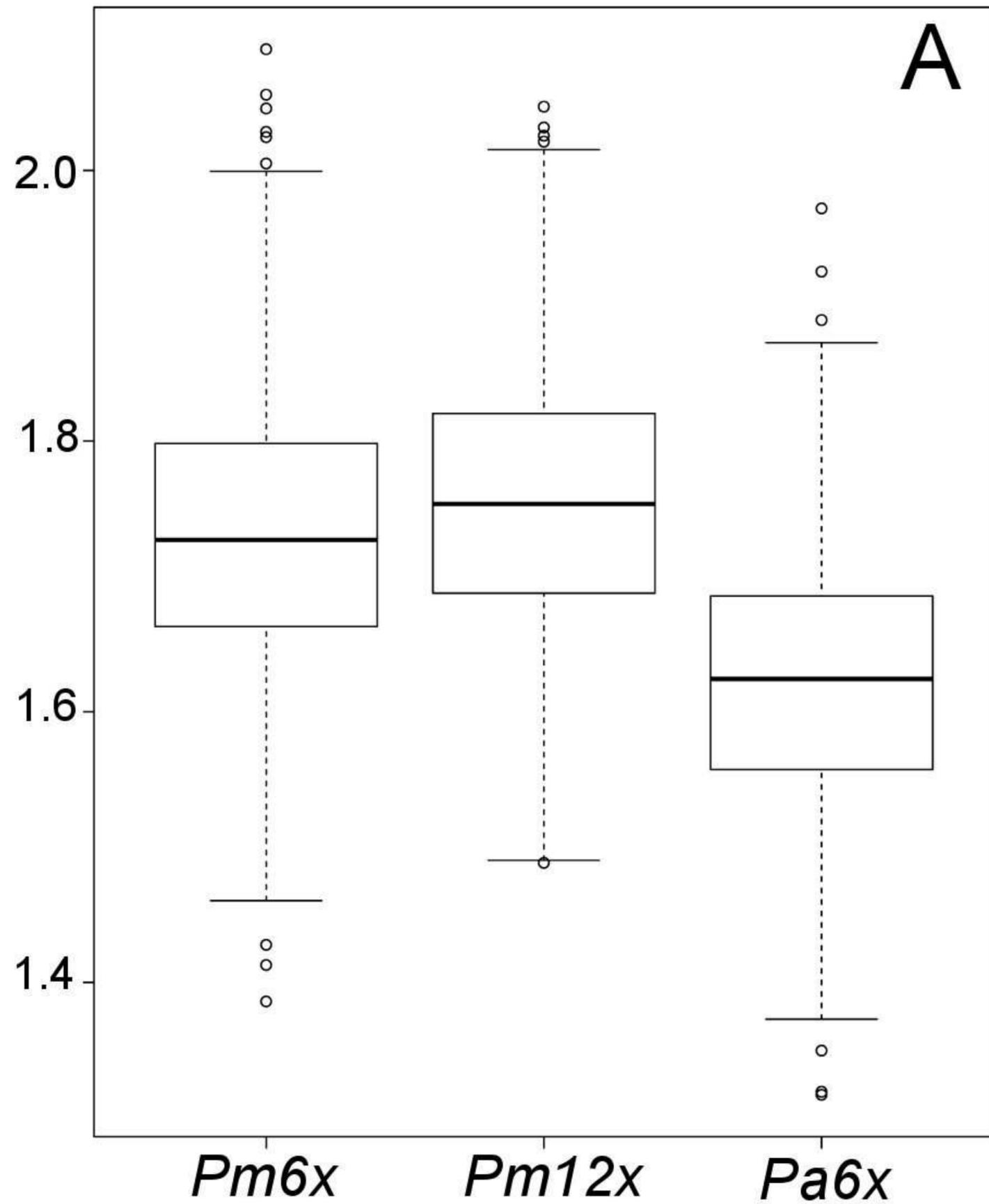


Pm12x vs Pa6x



PC1

PC2



SUPPORTING INFORMATION

Do floral and niche shifts favour the establishment and persistence of newly arisen polyploids? A case study in an Alpine primrose

CASAZZA Gabriele, BOUCHER Florian C., MINUTO Luigi, RANDIN Christophe and CONTI Elena

Table S1. Sampling of dodecaploids and hexaploids of *Primula marginata* and *Primula allionii*. Localities, coordinates (lat N, long E) of populations and the number of flowers (F) used in morphological analyses are reported.

Taxon	locality		lat N	long E	F
<i>P. allionii</i>	Traya, Val Roya, Fance	Tr	44°01'44"	7°34' 37"	51
	Chiarin, Val Roya, Fance	Ch	44°02'06"	7°33'34"	49
					100
<i>P. marginata</i> hexaploids	Chialvetta, Val Maira, Italy	CVT	44°26'56"	06°59'53"	12
	Chateaux de Queiras, Vallée di Queiras, France	CHQ	44°44'40"	06°46'11"	8
	Saint Auban, Vallée de l'Esteron, France	STB	43°51'16"	06°43'29"	15
	Mont Bruna, Vallée de l'Esteron, France	BRN	43°54'41"	07°14'15"	14
	Guillastre, , France	GUI	44°17'39"	07°09'43"	4
	L'Authion, Vallée da Bevera, France	THN	43°59'36"	07°25'36"	20
	Mont Baudion, Vallon de Paillon, France	MDB	43°48'02"	07°26'10"	18
	Cime de Grand Braus, Vallée da Bevera, France	BRS	43°53'27"	07°29'20"	17
	Beuil, M. Broussiere, Vallée du Var, France	BEU	44°06'00"	07°00'15"	12
					121
<i>P. marginata</i> dodecaploids	Rocca Barbena, Valle del Neva, Italy	RBR	44°09'28"	08°07'39"	27
	Cime du Bec Roux, Val Vermenagna, Italy	TND	44°09'15"	07°35'34"	14
	Monte Galero, Val Pennavaira, Italy	GLR	44°06'58"	07°55'30"	27
	Monte Saccarello, Valle Tanaro, Italy	SCC	44°03'41"	07°42'52"	28
	Val Cravina, Val Pesio, Italy	CRV	44°13'48"	07°37'58"	27
	Grotta del Bandito, Val Gesso, Italy	GBN	44°17'24"	07°25'44"	13
	Gorge de la Reina, Val Gesso, Italy	GRN	44°14'44"	07°25'41"	9
	Testa d'Alpe, Val Nervia, Italy	TTL	43°56'45"	07°33'28"	19
					164

Table S2. Sampling of dodecaploids (*Pm12x*) and hexaploids (*Pm6x*) of *Primula marginata* and *Primula allionii* (*Pa6x*) for populations, number of individuals, number of scapes, number of flower per scape and flower morphology.

Taxon	Populations	Individuals	Scapes	Flowers	Morph
<i>Pm12x</i>	8	56	80	164	80 L 84 S
<i>Pm6x</i>	9	49	78	121	47 L 74 S
<i>Pa6x</i>	2	18	-	100	48 L 52 S

Table S3. Results of Kruskal-Wallis test (K-W) to test whether each floral trait differed significantly between dodecaploids of *P. marginata* (*Pm12x*) and its closest relatives (hexaploids of *P. marginata*, *Pm6x* and *P. allionii*, *Pa6x*). The analysis was performed for long-styled (L) and for short-styled (S) morphs separately. Sample size and degree of freedom (df) was reported.

Response variable	Pair	Sample size	df	K-W		
				χ^2	<i>p</i> value	
L	number of flowers per scape	<i>Pm12x</i> vs <i>Pm6x</i>	47 vs 44	1	0.5942	0.4408
	petal lobe length	<i>Pm12x</i> vs <i>Pm6x</i>	76 vs 46	1	15.3416	<0.0001
		<i>Pm12x</i> vs <i>Pa6x</i>	76 vs 48	1	8.3947	0.0038
	corolla tube length	<i>Pm12x</i> vs <i>Pm6x</i>	80 vs 46	1	48.2629	<0.0001
		<i>Pm12x</i> vs <i>Pa6x</i>	80 vs 48	1	45.1379	<0.0001
	corolla mouth diameter	<i>Pm12x</i> vs <i>Pm6x</i>	80 vs 47	1	30.2619	<0.0001
		<i>Pm12x</i> vs <i>Pa6x</i>	80 vs 48	1	54.0492	<0.0001
	anther-stigma distance	<i>Pm12x</i> vs <i>Pm6x</i>	80 vs 46	1	53.5970	<0.0001
		<i>Pm12x</i> vs <i>Pa6x</i>	80 vs 48	1	37.484	<0.0001
S	number of flowers per scape	<i>Pm12x</i> vs <i>Pm6x</i>	47 vs 71	1	2.3944	0.1218
	petal lobe length	<i>Pm12x</i> vs <i>Pm6x</i>	77 vs 74	1	9.0198	0.0027
		<i>Pm12x</i> vs <i>Pa6x</i>	77 vs 52	1	0.2957	0.5866
	corolla tube length	<i>Pm12x</i> vs <i>Pm6x</i>	84 vs 74	1	29.7194	<0.0001
		<i>Pm12x</i> vs <i>Pa6x</i>	84 vs 52	1	46.1792	<0.0001
	corolla mouth diameter	<i>Pm12x</i> vs <i>Pm6x</i>	84 vs 74	1	29.7194	<0.0001
		<i>Pm12x</i> vs <i>Pa6x</i>	84 vs 52	1	43.2730	<0.0001
	anther-stigma distance	<i>Pm12x</i> vs <i>Pm6x</i>	84 vs 74	1	24.4100	<0.0001
		<i>Pm12x</i> vs <i>Pa6x</i>	84 vs 52	1	16.395	<0.0001

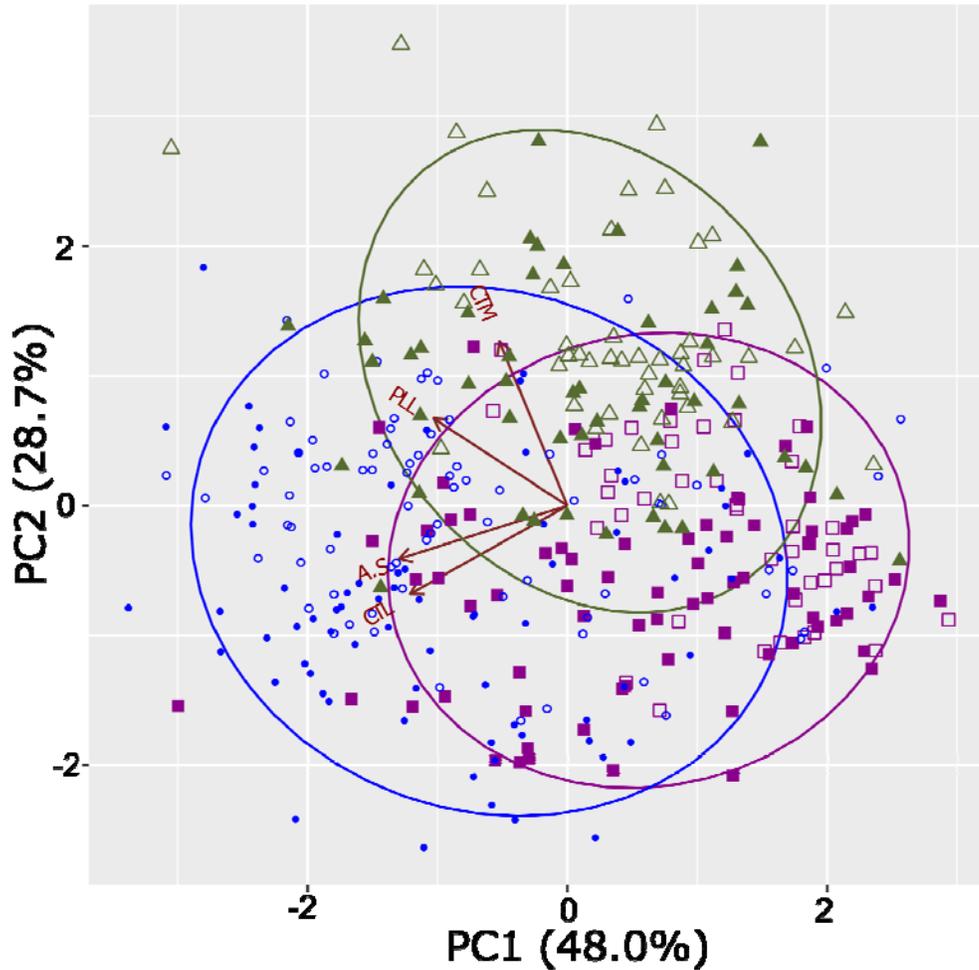


Figure S1. PCA of *P. marginata* hexaploids, *P. marginata* dodecaploids and *P. allionii* based on four floral traits. *P. marginata* hexaploids = magenta squares; *P. marginata* dodecaploids = blue circles; *P. allionii* = green triangles; long-style = empty forms; short-styled = filled forms. Ellipses represent 95% confidence interval. Petal lobe length = PLL, corolla tube length = CTL, corolla mouth diameter = CTM and anther-stigma distance = A.S.

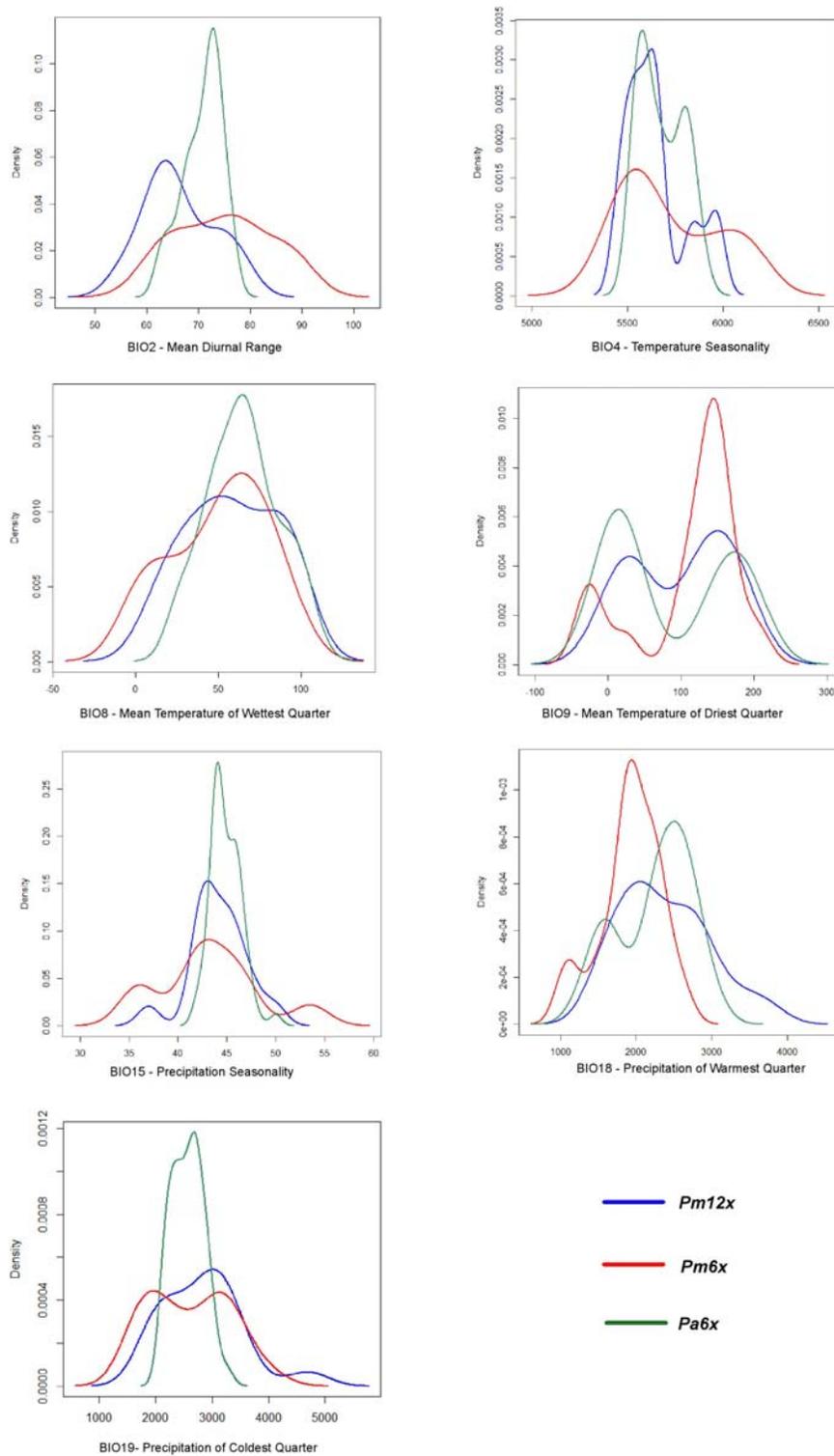


Figure S2. Kernel density plots of the five climatic variables at 100-m resolution for the two cytotypes of *P. marginata* and *P. allionii*. Blue line = dodecaploids (*Pm12x*), red line = hexaploids (*Pm6x*), green line = *P. allionii* (*Pa6x*).