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Is hybridization driving the evolution of climatic niche in *Alyssum montanum*?[1]

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PREMISE OF THE STUDY: After decades of interest, the contribution of hybridization to ecological diversification remains unclear. Hybridization is a potent source of novelty, but nascent hybrid lineages must overcome reproductive and ecological competition from their parental species. Here, we assess whether hybrid speciation is advantageous over alternative modes of speciation, by comparing the geographical and ecological ranges and climatic niche evolutionary rates of stabilized allopolyploid vs. autopolyploids in the *Alyssum montanum* species complex.

METHODS: We combined an extensive review of studies addressing the systematics and genetic diversity of *A. montanum* s.l., with flow cytometry and cloning of nuclear markers, to establish the ploidy level and putative hybrid nature of 205 populations. The respective geographic distribution and climatic niche evolution dynamics of the allo- and autopolyploids were investigated using multivariate analyses and comparative phylogenetic approaches.

KEY RESULTS: As expected by theory, allopolyploids occur mainly along contact zones and are generally spatially overlapping with their diploid counterparts. However, they demonstrate higher rates of niche evolution and expand into different climatic conditions than those of their diploid congeners. In contrast, autopolyploids show lower rates of niche evolution, occupy ecological niches similar to their ancestors and are restricted to less competitive and peripheral geographic areas.

CONCLUSIONS: Hybridization thus seems advantageous by promoting ecological niche evolution and more readily allowing escape from competitive exclusion.

KEY WORDS allopolyploidy; autopolyploidy; Brassicaceae; competition; diversification; ecological novelty; local adaptation; minority cytotype disadvantage; transgressive segregation

Understanding the drivers of phenotypic variation within species is of major importance to identify the processes that generate ecological diversity and enable local adaptation (Darwin, 1859). Several sources of diversity on which natural selection acts have been classically considered, including mutations and the reshuffling of standing variation via migration and recombination (Ridley, 2003). In this framework, the importance of hybridization as a driver of ecological diversification has long been debated. Whereas hybrids were viewed primarily as unfit or maladaptive by promoters of the biological species concept (Mayr, 1942), hybridization has been progressively recognized as an important source of ecological novelty via character segregation (Stebbins, 1959; Arnold et al., 2012; and references therein), introgression of traits among species (Anderson, 1949; Lewontin and Birch, 1966; Rieseberg, 2009; Whitney et al., 2015), or as a potential source of new species (i.e., "hybrid speciation"; Stebbins, 1959; Rieseberg, 1997; Vallejo-Marín et al., 2015).

Numerous studies have recorded the genetic footprints of hybridization and introgression events in plant and animal groups (e.g., Mallet, 2005; Whitney et al., 2010) and have shown that reticulations occur throughout phylogenies of many lineages (e.g.,

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Seehausen, 2004; Lai et al., 2012). From a population biology perspective, most studies exploring the dynamics and establishment of hybrid species have targeted homoploid speciation (i.e., at the diploid level). Such hybrid species are, however, relatively difficult to detect, with only ca. 20 well-known examples described so far, mostly in plants (Yakimowski and Rieseberg, 2014). Surprisingly, the large body of literature about plant polyploids has largely been underexploited (but see Stebbins, 1985) to assess the relative benefits of hybridization in evolution (Ramsey and Ramsey, 2014). Polyploidy, or whole-genome doubling, has long been recognized as a frequent mode of sympatric speciation in plants (Arrigo and Barker, 2012; Soltis et al., 2014); this phenomenon is however much rarer in animals (Mable, 2004). Polyploids necessarily originate within diploid populations, from which they are at least partially reproductively isolated due to differing chromosome numbers, leading to meiotic abnormalities, unbalanced gene dosage, and increased autogamy (Arrigo and Baker, 2012; Soltis et al., 2014). Polyploidization can arise either within the same genetic lineage—leading to autopolyploids—or in areas of species or lineage overlap—leading to allopolyploids, which represent a large fraction of stabilized plant hybrid species. Also, because plants produce an average of 0.465% of unreduced gametes at each generation (Ramsey and Schemske, 1998), autopolyploid genotypes originate frequently in diploid populations and are expected to form at higher rates than allopolyploids (Ramsey and Schemske, 1998). Allo- and autopolyploids do not differ for basic fitness components such as the proportion of unbalanced gametes and/or progenies they produce and show similar levels of pollen and seed fertility (Ramsey and Schemske, 2002). A striking difference between both ploidy types lies, theoretically, in the expression of differing levels of phenotypic variation, being intrinsically higher in the allopolyploids. Accordingly, hybridization is generally associated with larger gene expression alterations than those arising solely after whole genome doubling (e.g., see Levin, 1983; Soltis et al., 2014; Yoo et al., 2014) and often leads to transgressive segregation (i.e., where hybrids display trait values exceeding those of their parental species; Washburn and Birchler, 2014). In addition, increased phenotypic variation is expected in allopolyploid progenies because homoeologous chromosomes (i.e., those originating from distinct parental genomes) show varying levels of recombination and polysomic inheritance (Ramsey and Schemske, 2002) and promote the segregation of parental characters. Those high and low levels of phenotypic variation between allo- and autopolyploid lineages should respectively translate into varying abilities to occupy alternative ecological niches and reveal contrasted adaptive perspectives, with the highest rates of niche evolution being expected for the allopolypoids. The sympatric nature of polyploid speciation should further exacerbate these differences because both allo- and autopolyploids arise within established diploid (and polyploid) populations and suffer from "minority cytotype exclusion" (i.e., the low-frequency cytotype faces both ecological and reproductive competition from the dominant cytotype, Levin, 1983). Hence, those polyploids showing the largest phenotypic variation might have better opportunities to establish new populations and escape competitive challenges from their diploid relatives.

Several studies have recently assessed the impact of polyploidy on the evolution of ecological niches (e.g., McIntyre, 2012; Laport et al., 2013; Glennon et al., 2014; Harbert et al., 2014; Thompson et al., 2014). However, to the best of our knowledge, and as outlined recently (Ramsey and Ramsey, 2014), no study has directly compared the ecological and geographical niches between allo- and autopolyploids in a given group to assess whether hybridization provides any selective benefit in the context of polyploid speciation. Here, we contribute to answering this question by focusing on *Alyssum montanum* s.l., a polyploid complex spanning a wide ecological and geographical range in the West-Palearctic. On the basis of an extensive review of the literature addressing the taxonomy and molecular genetics of *Alyssum montanum* s.l., we tentatively identified the ploidy status and hybrid nature of 205 populations, and examined differences in the climatic niche between allopolyploids and autopolyploids.

Using quantitative ecology and comparative phylogenetic approaches, we tested the following hypotheses: (1) allopolyploids expand over a larger ecological niche than their autopolyploid counterparts, and (2) the allopolyploids might readily escape from minority cytotype exclusion and therefore establish at closer geographical proximity to their diploid counterparts. (3) In contrast, we expect autopolyploids to explore an ecological niche much similar to that of their diploid counterparts, and (4) the autopolyploids would essentially exist in geographical areas offering reduced levels of competitive exclusion.

MATERIALS AND METHODS

Data collection and assignment to allo- or autopolyploids—*Alyssum montanum* s.l. is a monophyletic polyploid complex (Rešetnik et al., 2013) within the Brassicaceae, including over 30 species and subspecies with variable levels of ploidy. This group has diversified over the last 2 million years (Li et al., 2014) and comprises perennial species distributed in Europe, western Asia, and northern Africa, with maximal species diversity in submediterranean areas (Jalas et al., 1996; Španiel et al., 2011a, b; Zozomová-Lihová et al., 2014; and references therein). Here, we extensively reviewed the recent literature addressing the taxonomy and molecular genetics of *Alyssum montanum* s.l. (Španiel et al., 2011a, b, 2012a, b, 2015; Zozomová-Lihová et al., 2014; and references therein) to assemble a georeferenced database of 205 populations spanning the major part of the geographical range of the group (Fig. 1). The taxonomic status (at the species and subspecies levels), the geographical coordinates, and ploidy levels were recorded for each surveyed population. In particular, DNA ploidy levels were estimated by flow cytometry and calibrated by chromosome counting of a few individuals of each ploidy level (for details, see Španiel et al., 2011a). Most of the ploidy level records were published in previous studies (Španiel et al., 2011a, b, 2012a; Zozomová-Lihová et al., 2014; Magauer et al., 2014), and the rest are presented here as new records (Appendix S1 in the online Supplemental Data of this article). With the exception of complex and so far insufficiently resolved species diversity and evolutionary history in the Balkans (S. Španiel et al., unpublished data), the nature of most polyploids from the other European areas could be established using molecular studies (i.e., AFLPs, sequencing of chloroplast regions and ITS clones; see references above and Appendix S1).

We classified each polyploid population as either allo- or autopolyploids, based on available molecular evidence (summarized in Appendix S1). Such assignments may be difficult in practice, because allo- and autopolyploids represent the extremes of a

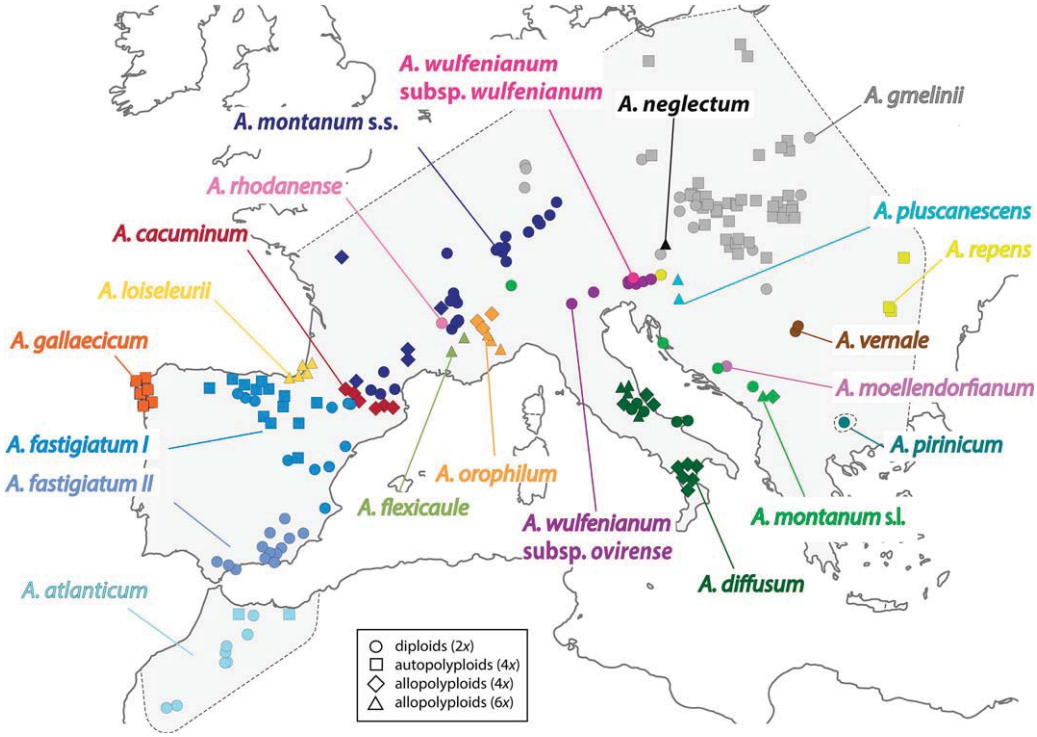

FIGURE 1 Overview of the 205 populations included in our survey, based on a georeferenced database addressing the taxonomy and molecular genetics of 96 diploid, 49 hybrid allopolyploid, and 60 autopolyploid populations belonging to the *Alyssum montanum* s.l. species complex (from Španiel et al., 2011a, b, 2012a, b; Zozomová-Lihová et al., 2014; and references therein; see further details in online Appendix S1). "*A. fastigiatum II*" stands for the Cantabrian phylogeographic group reported in *A. fastigiatum* (as in Zozomová-Lihová et al., 2014). The complete geographical range of *Alyssum montanum* s.l., as in Atlas Florae Europaeae (Jalas et al., 1996), is delineated with a dashed line.

divergence continuum between the parental diploid genome(s) from which they derive (Tayalé and Parisod, 2013). In addition, the systematic treatment of polyploid complexes is generally obscured by ongoing lineage sorting and reticulation events that further complicate the use of taxonomy-based criteria. For instance, we could not directly refer to "inter-" or "intra-specific" levels, which are usually used to discriminate allopolyploids from autopolyploids (as suggested by Ramsey and Schemske, 1998). For this reason, we further specify the definitions that were used here. Strictly speaking, autopolyploidy arises from whole-genome doubling events at the intralineage level (Ramsey and Schemske, 1998). As a result, autopolyploids combine essentially nondifferentiated genomes and bear highly similar homoeologous chromosomes (e.g., AAAA). In contrast, allopolyploidy refers to any whole-genome doubling event following interlineage hybridization (Ramsey and Schemske, 1998). As a result, allopolyploids combine differentiated parental genomes and distinct homoeologous chromosomes (e.g., AABB). Here, we assign populations to allo- or autopolyploids according to the available evidence of divergence among their parental genomes (following Stebbins, 1985), irrespectively of taxonomic treatment (Appendix S1 summarizes every population assignment in line with available evidence). Accordingly, the consistent presence of multiple differentiated copies of nuclear genes within a specimen (assessed via cloning and sequencing of the internal transcribed spacer using primers and PCR conditions of Magauer et al. [2014], GenBank accessions KP015390 to KP015487; see also Appendix S1), the patterns of reticulation, and the presence of chloroplast haplotypes diagnostic of recurrent polyploidy involving distinct maternal lineages within a species (or group of populations) were all taken here as diagnostic of hybrid origins for the focal polyploids (we acknowledge that a few instances of admixture events might be inferred as allopolyploidies using those criteria). In contrast, populations showing the absence of such diagnostic signals were scored as autopolyploids. Our survey thus included 44 and 49 populations of allo- and autopolyploids, respectively (Appendix S1), as well as 96 diploid populations. The allopoyploids included 30 tetraploid and 14 hexaploid populations that were analyzed irrespectively of ploidy level to maintain sufficient statistical power (i.e., excluding the hexaploids decreases power without altering our conclusions; see further details in Appendix S2 in the online Supplemental Data). In addition, the origin of another 16 polyploid populations (i.e., mostly from highly diversified *A. repens* and *A. fastigiatum* populations of northeastern Spain and Cantabria; see Appendix S1) remained uncertain. These populations were nevertheless included in our analyses as tentative autopolyploids because this is the most parsimonious source of polyploidy (Barker et al., 2015; nearly identical results were obtained when treating these populations as allopolyploids [see results in Appendix S2]).

Ecological niche and differentiation between polyploids and diploids—We estimated the climatic niche of the 205 populations with 19 Bioclim variables (Hijmans et al., 2005; online Appendix S3) at a 2.5-min resolution using the program QGIS V 2.8 (QuantumGIS Development Team, 2014). We completed this data set with 1000 data points sampled randomly from the expected distribution of *A. montanum* s.l. (following Atlas Florae Europaeae; Jalas et al., 1996), to consistently place our population sampling into the complete climatic space occupied by the species complex. We then standardized the obtained climatic variables (mean subtraction followed by variance division) and used these for computing Euclidean distances among populations. First, we summarized these distances with a principal component analysis (based on a correlation matrix), into two eigenaxes PCA1 and PCA2 (determining the number of representative eigenaxes with the broken-stick model approach; Legendre and Legendre, 1998). We considered these eigenaxes as climatic proxies in the comparative phylogenetic analyses (see below). We then assessed the levels of niche overlap

between the diploid and polyploid populations by using an approach described by Petitpierre et al. (2012) and implemented in the ecospat V 1.1 package (Petitpierre et al., 2012). Briefly, the PCA1 and PCA2 eigenaxes were binned over an environmental grid of 300 × 300 cells. The abundance of diploid, allopolyploid, and autopolyploid populations were computed for each grid cell and smoothed using a density kernel. The obtained densities were then used as proxies of the population abundances over the environmental space and used to compute (1) the Schoener's *D* (a metric ranging from 0 to 1 directly proportional to the degree of niche overlap among the compared cytotypes) and its associated niche equivalence test (a simple random permutation procedure among the compared cytotypes), (2) the niche stability of polyploids (i.e., as the proportion of polyploid densities occurring in the diploid niche), (3) niche expansion of polyploids (i.e., as the proportion of polyploid populations exploring alternative niches), and (4) diploid niche unfilling, as the proportion of the diploid niche that is void of polyploids.

Finally, we assessed how much allo- and autopolyploids differed climatically from diploids, as a function of geographic distance (used here as a proxy of relatedness). To this end, we compared each polyploid population to the diploids occurring within a 1000 km perimeter and checked how their corresponding climatic and geographic distances were related. Following preliminary analyses, we modeled this relation with a power function, $y = ax^b$, where y is the climatic distance between the compared populations, x is the corresponding geographical distance, and a and b are model parameters to be estimated. We then used nonlinear least-squares (using the nls function in R CRAN (R Core Team, 2014) included in the stats package) to estimate the respective model parameters of allo- and autopolyploids. These estimations followed a resampling procedure aimed at (1) correcting for unequal sampling between allo- and autopolyploids (i.e., using 44 populations of each cytotype to compute distances) and (2) avoiding pseudoreplication by allowing each diploid population to be included in only one instance of nonlinear least squares. We computed the final estimates as medians of parameters obtained from 1000 resampling instances. We tested the significance levels between allo- and autopolyploids using nonparametric Wilcoxon rank-sum tests.

Comparative phylogenetics—The distance-based methods described above considered our 205 populations as independent data points. Populations are genetically related to each other, and a phylogenetic framework is required to properly compare the rates of climatic niche evolution of allo- and autopolyploids. To this end, we sequenced cpDNA regions [*rpl32-trnL*(UAG) intergenic region and the *rpoB-trnC* intergenic spacer, GenBank accessions KP015199 to KP015389, Appendix S1] using the PCR conditions of Zozomová-Lihová et al. (2014) and collected publicly available sequences for those regions to produce a data set of 281 *A. montanum* s.l. specimens (GenBank, as of 17 June 2014), covering a subset of 130 populations among those included in our database. We aligned these sequences with the program MUSCLE v. 3.8.31 (Edgar, 2004), resulting in alignments that we further refined manually (using BioEdit; Hall, 1999). We treated the aligned *rpl32-trnL*(UAG) and *rpoB-trnC* data sets, as well as their corresponding gaps as separate partitions in phylogenetic inferences. We selected the best fitting models of evolution for each partition with the program MrModel test v. 2.3 (Nylander, 2004; resulting in GTR+GAMMA for all), and declared gaps (recoded as presence–absence data using the program FastGap v. 1.2; Borchsenius, 2009) as restriction sites. We performed the phylogenetic inferences in the program MrBayes V 3.2.1 (Ronquist and Huelsenbeck, 2003) using four parallel runs, 20,000,000 generation long and four Markov chain Monte Carlo (MCMC) chains, with parameters sampled every 1000 steps. We assessed the convergence with the Gelman–Rubin criterion and upon visual inspection of posterior traces (using Tracer v. 1.6; Rambaut et al., 2014). After discarding the initial 30% of each run as burn-in, we combined the four analyses in a single Bayesian posterior distribution from which we randomly selected 1000 trees. Finally, we randomly picked one specimen per population in every selected tree before investigating patterns of climatic niche evolution (this thinning procedure was used to match our specimen-level phylogenies with the ecological and ploidy data sets; see online Appendices S4 and S5 for an illustration of the complete procedure).

We investigated the respective rates of climatic niche evolution for the allo- and autopolyploids using Brownie (using methods implemented in the R CRAN package phytools v. 0.4-45; O'Meara et al., 2006; Revell, 2012). According to a set of preliminary analyses, this framework is appropriate for analyzing allopolyploid lineages with maternally inherited markers (see Appendix S4). Briefly, this approach models the evolution of a continuous trait (here: the PCA1 / PCA2 eigenaxes defined earlier) along a phylogeny, by fitting Brownian motion processes to a set of lineages of interest (here: diploids, allo-, and autopolyploids). The algorithm then estimates variance parameters depicting the rates of trait evolution for every considered lineage (here: $\lambda_{diploid}$, λ_{allo} and λ_{auto}). Brownie requires every branch of the phylogeny to be assigned to a given lineage. To this end, we reconstructed ancestral ploidy states using maximum likelihood methods implemented in the "ape" R CRAN package v. 3.2 (Pagel, 1994; Paradis et al., 2004), by considering the cytotype state at the tips and assuming a simple transition matrix allowing shifts from diploid to allo- / autopolyploids (reversions to the diploid state were not allowed, following the method of Ramsey and Schemske [2002]; also, transitions among allo- and autopolyploidy were excluded for reasons of parsimony). We considered three Brownie models by assuming either (1) a unique rate of evolution for the complete phylogeny (H0), (2) distinct rates for the diploids and polyploids (i.e., allo- and autopolyploids shared the same rate, H1), or (3) distinct rates for the diploids, allo- and autopolyploids (H2). We selected the best model according to the AIC criterion (O'Meara et al., 2006). Finally, we accounted for phylogenetic uncertainty by repeating the complete procedure over each of the 1000 randomly sampled trees. We considered the final results of the 1000 Brownie instances by (1) recording the number of trees supporting the H0, H1, and H2 models and (2) summarizing the respective Brownian variances yielded by the diploid, allo-, and autopolyploids (using variance ratios, as $\lambda_{diploid} / \lambda_{allo}$, $\lambda_{diploid} / \lambda_{auto}$, and $\lambda_{allo} / \lambda_{auto}$). Significance levels were assessed using two permutation procedures by which either the ploidy levels (R.ploidy) or the PCA1 / PCA2 eigenvalues (R.clim) were reassigned randomly (without replacement) to the phylogeny before reapplying the Brownie analysis. The outputs of those permutations were compared with the empirical results using nonparametric Kruskal–Wallis tests (1000 permutations, applying a Dunn post hoc test) and correcting for multiple testing (Bonferroni *p*-value adjustment).

RESULTS

The allo- and autopolyploids differed markedly in terms of geographical and climatic ranges. The allopolyploids occurred throughout the distribution of diploids (Fig. 2) and prevailed in mountain ranges such as the Pyrenees, the southwestern Alps, and the Apennines (Fig. 2B), i.e., in geographical areas characterized by fine-grained and heterogeneous microclimatic conditions (see regional climatic variances in online Appendix S6). In contrast, the autopolyploids were mostly recorded in peripheral areas—when considering the complete species complex—and were especially prevalent in northern Spain and Central Europe (Fig. 2C), i.e., in areas with more homogeneous climatic characteristics (Appendix S6).

Overall, the allo- and autopolyploids occupied climatic niches that were statistically different from that of the diploids, with $D_{allo} = 0.22$ ($p = 0.02$) and $D_{auto} = 0.17$ ($p = 0.02$). The allopolyploids appeared as more interspersed within the overall climatic space of the diploids (72% of niche overlap, Fig. 2D) than were the autopolyploids (62% of overlap). Both allo- and autopolyploids explored climatic niches out of those occupied by the diploids. Accordingly, 28% of the allopolyploids expanded in climatic conditions void of diploids such as coastal dune habitats (i.e., *A. loiseleurii* populations from southwestern France) and humid environments (populations labeled ① and ② in Fig. 2B, D). Likewise, 38% of the autopolyploids were found in ecological niches that were either completely innovative (*A. gallaecicum*, from northwestern Spain, occupies a niche similar to the *A. loiseleurii* allopolyploids: populations ③ in Fig. 2C, D), or only marginally occupied by the diploids (e.g., *A. gmelinii*, from Central Europe, is dominated by polyploids that otherwise have a niche similar to their diploid relatives: populations ④ in Fig. 2C, D). Finally, allo- and autopolyploids differed in terms of diploid niche unfilling, as 31% and 41% of the diploid climatic space was not occupied by the allo- and autopolyploids, respectively. Consistent with these results, allo- and autopolyploids differed in terms of ecological differentiation with the diploids, yet at fine spatial scales. Accordingly, the allopolyploids generally expanded in climatic niches that were distinct from those occupied by their geographically closest diploid neighbours (Fig. 2E, F). In contrast, the autopolyploids tended to expand into climatic niches similar to their immediate diploid neighbours (Fig. 2E, F). This difference was supported by nonlinear models that yielded significantly different parameter values for the allo- and autopolyploids (with power functions of $y_{allo} = 2.79x_{allo}^{0.13}$ and $y_{auto} = 0.47x_{auto}^{0.36}$).

Analyses of the rates of trait evolution further supported these results, while explicitly accounting for genetic relatedness among the compared populations. Overall, most phylogenies sampled from the Bayesian posterior distribution supported the H2 model (with 77% and 39% of support, for PCA1 and PCA2, respectively), suggesting that the climatic niches of diploids, allo- and autopolyploids were evolving under different rates (Fig. 3A, B). These results were further supported by the permutation procedures, showing that random ploidy levels or climatic values could not yield comparable patterns. The allopolyploids had significantly enhanced rates of climatic niche evolution relative to the diploids (that followed a Brownian motion process ca. 4 times less variable than that of allopolyploids) and the autopolyploids (ca. 10 times less variable than allopolyploids). Finally, the ecological niche of autopolyploids appeared as evolving significantly slower (at rates ca. 2.5 times lower) than those of the diploids (Fig. 3C, D).

DISCUSSION

As long hypothesized by experimental studies (Levin, 1983; Ramsey and Schemske, 2002; Ramsey and Ramsey, 2014), we found that the allo- and autopolyploid populations of the *Alyssum montanum* s.l. species complex differed markedly in their climatic and geographical ranges. Our results showed that allopolyploids explored climatic niches that differed from those of their closest geographical and genetic diploid relatives. As a result, the allopolyploid populations showed high rates of climatic niche evolution and could establish in a climatic space largely overlapping with that of the diploids. In contrast, the autopolyploids tended to explore niches more similar to those of their closest geographical and genetic diploid counterparts. As a result, the autopolyploids showed lower climatic rates of evolution and remained confined to peripheral areas and ecological niches.

Different geographical distributions of allo- vs. autopolyploids—The majority of allopolyploids occurred in mountain ranges corresponding to well-identified phylogeographic suture zones (Hewitt, 2011). These areas shaped the current genetic structure of the *Alyssum montanum* s.l. species complex (Zozomová-Lihová et al., 2014) by acting as dispersal barriers and limiting exchanges among lineages originating from distinct glacial refugia (e.g., *A. montanum* s.s. and *A. fastigiatum* did not cross the Pyrenees; Španiel et al., 2011b; Zozomová-Lihová et al., 2014). As a consequence, these mountain massifs now appear as contact zones among genetically differentiated groups and represent the cradle of most allopolyploids observed in the group. This expected pattern confirms that secondary contacts, occurring at the range margins of previously differentiated genetic lineages, are the major source of allopolyploids (Stebbins, 1985; Hewitt, 2011; Abbott et al., 2013).

Contrasting with the observation that polyploids arise frequently, and in virtually any diploid species (owing to the frequent formation of unreduced gametes; Ramsey and Schemske, 1998), our analyses showed that the autopolyploids were absent from the distribution center of the *A. montanum* s.l. species complex and rather occupied peripheral areas and ecological niches (autopolyploids were absent from about 41% of the ecological space occupied by their diploid relatives). The presence of autopolyploids was rather expected in central areas because they arise frequently and have, at least in mountain ranges, as many opportunities as the allopolyploids to explore alternative microclimatic niches. Several hypotheses might explain this result. Autopolyploids might simply fail to establish new populations when challenged by competitive exclusion in an environment dominated by diploids (Levin, 1983). Under such a scenario, autopolyploids might better subsist in areas offering increased establishment opportunities. Postglacial expansion ranges, as, for instance, in Central Europe, may provide such environments that sustain numerous founding events and often allow the fixation of rare variants (polyploid establishment might well follow a process similar to "allele surfing", where neutral or even deleterious mutations get fixed during range expansions owing to purely demographic effects; Excoffier and Ray, 2008). The scarcity of autopolyploids in the distribution center of

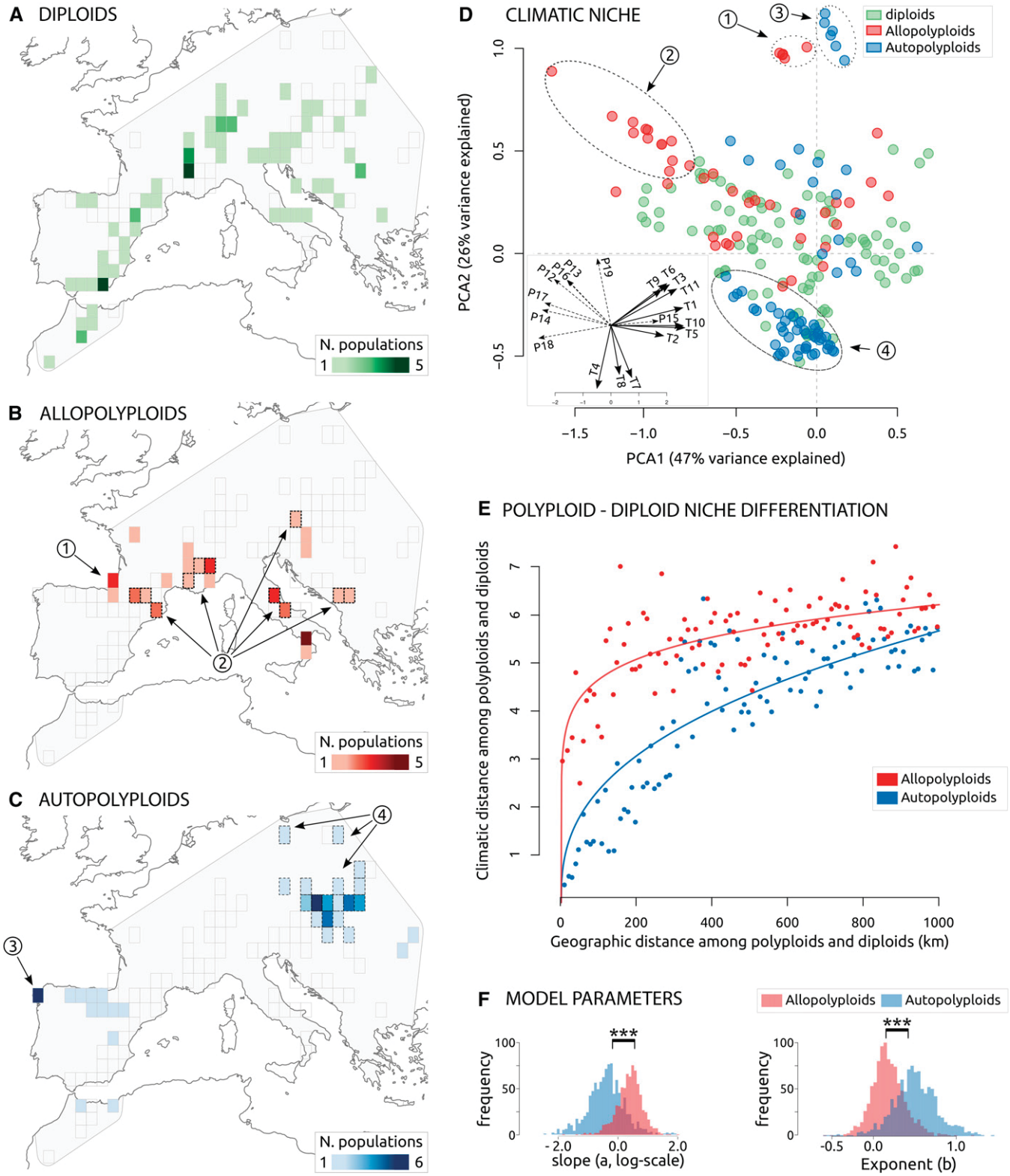

FIGURE 2 Geographic and climatic features of *Alyssum montanum* s.l. polyploids. (A–C) Geographical distribution of diploids, allopolyploids, and autopolyploids. The 205 populations surveyed in the current study were assigned to 1-degree grid cells and counted to estimate the local prevalence of each cytotype. The complete geographical range of *Alyssum montanum* s.l., as in Atlas Florae Europaeae (Jalas et al., 1996), is delineated with a dashed line. (D) Principal component analysis based on 19 standardized Bioclim variables (T1–T11: temperature descriptors 1–11, P12–P19: precipitation descriptors 12–19; Hijmans et al., 2005), aimed at placing the 205 surveyed populations into the complete climatic space occupied by *Alyssum*

the species complex could also relate to the criteria used while inferring the polyploid origin of the investigated populations (i.e., evidences of hybridization). Indeed, autopolyploids are essentially absent from phylogeographic suture zones. However, the diploid populations occurring in these areas might show an increased admixture baseline, and in turn, their polyploid descendants would preferentially be assigned a hybrid origin. Similarly, we acknowledge that admixture events among (auto)polyploids might appear as allopolyploidies using our criteria. This particular explanation might hold, for instance, for the 16 polyploid populations of unclear origin that we mentioned earlier. From a practical standpoint, however, we do not expect such misclassifications to significantly affect our conclusions because they would underestimate the general levels of climatic variation associated to allopolyploids, which is a conservative bias.

Hybridization as a source of ecological novelty and evolutionary success?—Although admittedly focusing on climatic factors and ignoring additional components of the ecological niche (e.g., soil composition and interspecific interactions), our results are compatible with hybridization acting as a source of adaptive novelty, with the allopolyploids displaying increased rates of niche evolution compared with the autopolyploids and the diploids. As a result, the allopolyploids appear as more prone than their autopolyploid counterparts to escape from competitive challenges by establishing into alternative and locally vacant ecological niches and can therefore exist closer to their parental species. In contrast, the autopolyploids have lower rates of climatic niche evolution compared with their diploid congeners (but see *A. gallaecicum*). This result suggests that the autopolyploids essentially remain within the climatic niche of their diploid counterparts and might not therefore escape from competitive exclusion. This pattern is notably in line with a recent survey of 20 plant species, showing that most neopolyploids were expanding within the climatic niche of their diploid counterparts (Glennon et al., 2014).

Several adaptive outcomes are expected from hybridization events. Perhaps the most immediate benefit lies in reshuffling the genetic diversity fixed in parental lineages (Seehausen, 2004; Arnold et al., 2012; Abbott et al., 2013) to produce the phenotypic variation needed for exploring alternative niches. Accordingly, allopolyploids typically exhibit varying numbers of bivalent vs. multivalent chromosome pairings during meiosis (as a function of genetic differentiation among the parental genomes; Ramsey and Schemske, 2002). Over the longer term, this generates variation—via character segregation—at faster rates than most mutational processes (Anderson, 1949; Lewontin and Birch, 1966; Ramsey and Schemske, 2002; Abbott et al., 2013), at least in situations where the parental species occupy distinct adaptive ranges and when their divergence results from an allopatric barrier to gene flow or any corresponding genome-wide reproductive barrier such as polyploidy (Barton, 2013). In addition, allopolyploids, in contrast to their autopolyploid counterparts, often display "genome shock syndromes" that involve alterations of metabolic and transcriptomic interactions (e.g., Buggs et al., 2014) along with extensive genome reorganizations (e.g., via bursts of transposition events and changes in gene content, copy number, and chromosome structure; Tayalé and Parisod, 2013). These mechanisms might further contribute to generate, aside from many unfit genomic combinations (Mayr, 1942; Stebbins, 1959), the phenotypic variation needed to explore environmental heterogeneity. Our results suggest that allopolyploids benefit from increased ecological variation and are especially prone to colonize novel habitats (as observed by Stebbins, 1985; Brochmann and Brysting, 2004). Eventually, our results are in line with earlier studies documenting ecological niche shifts and adaptive radiations associated with hybridization events, both in plant and animal systems (e.g., Arnold et al., 2012; Lewontin and Birch, 1966; Stebbins, 1985; Seehausen, 2004; including invasive species, Hovick et al., 2012).

Finally, our results suggest that allo- and autopolyploids could differ in their diversification rates. Hybridization may favor speciation events, thanks to the diversity boost it introduces by reshuffling the genetic diversity fixed in parental lineages (see Arnold et al., 2012; Abbott et al., 2013), followed by the radiation of alternative hybrid genotypes into distinct ecological niches (e.g., Seehausen, 2004; Abbott et al., 2013). In addition, allo- and autopolyploids might differ in extinction probabilities, with increased extinction rates being expected for the autopolyploids, because they are likely to face competitive exclusion from the diploids over the long term (Barker et al., 2015). In contrast, allopolyploids may be associated with lower probability of extinction because they readily escape from competitive challenges.

CONCLUSION

Our study gives full credit to far-sighted studies that had outlined hybridization as a potent source of variation fuelling local adaptation processes (Anderson, 1949; Stebbins, 1959; Lewontin and Birch, 1966). The observed results fit within theoretical expectations about hybridization, showing that allopolyploids experience

←

montanum s.l. The explained variance as well as the contribution of each climatic variable (temperature and precipitation descriptors figured as continuous and dashed lines, respectively; see also Appendix S3) to the first two eigenaxes (PCA1 and PCA2) are indicated. (E) Climatic vs. geographic Euclidean distances between the allo- / autopolyploids and the diploids. We compared the levels of climatic differentiation between the polyploid and the diploid populations as a function of geographic distance. This relation is modeled with $y = a \cdot x^b$, where y is the climatic distance between the compared populations, x is the corresponding geographical distance, and a and b are model parameters estimated using nonlinear least-squares. The complete analysis considered more than 38,000 pairwise distances among polyploid and diploid populations (with 1000 km as the upper bound). These are summarized here for readability purposes, by applying medians over a 10-km sliding window (median values displayed as blue and red dots). The fitted models are displayed as blue and red curves. (F) Corresponding model parameters estimated for allo- and autopolyploids. Significance levels were estimated using Wilcoxon rank-sum tests (***$P < 0.001$). (A–D) The geographical location and climatic niches of several polyploid populations of interest are highlighted by arrows and ellipses: ① and ③ *A. gallaecicum* and *A. loiseleurii*, two polyploid species demonstrating a case of convergent ecological shift (Zozomová-Lihová et al., 2014), ② allopolyploids expanding into climatic niches (characterized by increased humidity) that were not occupied by their diploid counterparts, ④ autopolyploids in *A. gmelinii*.

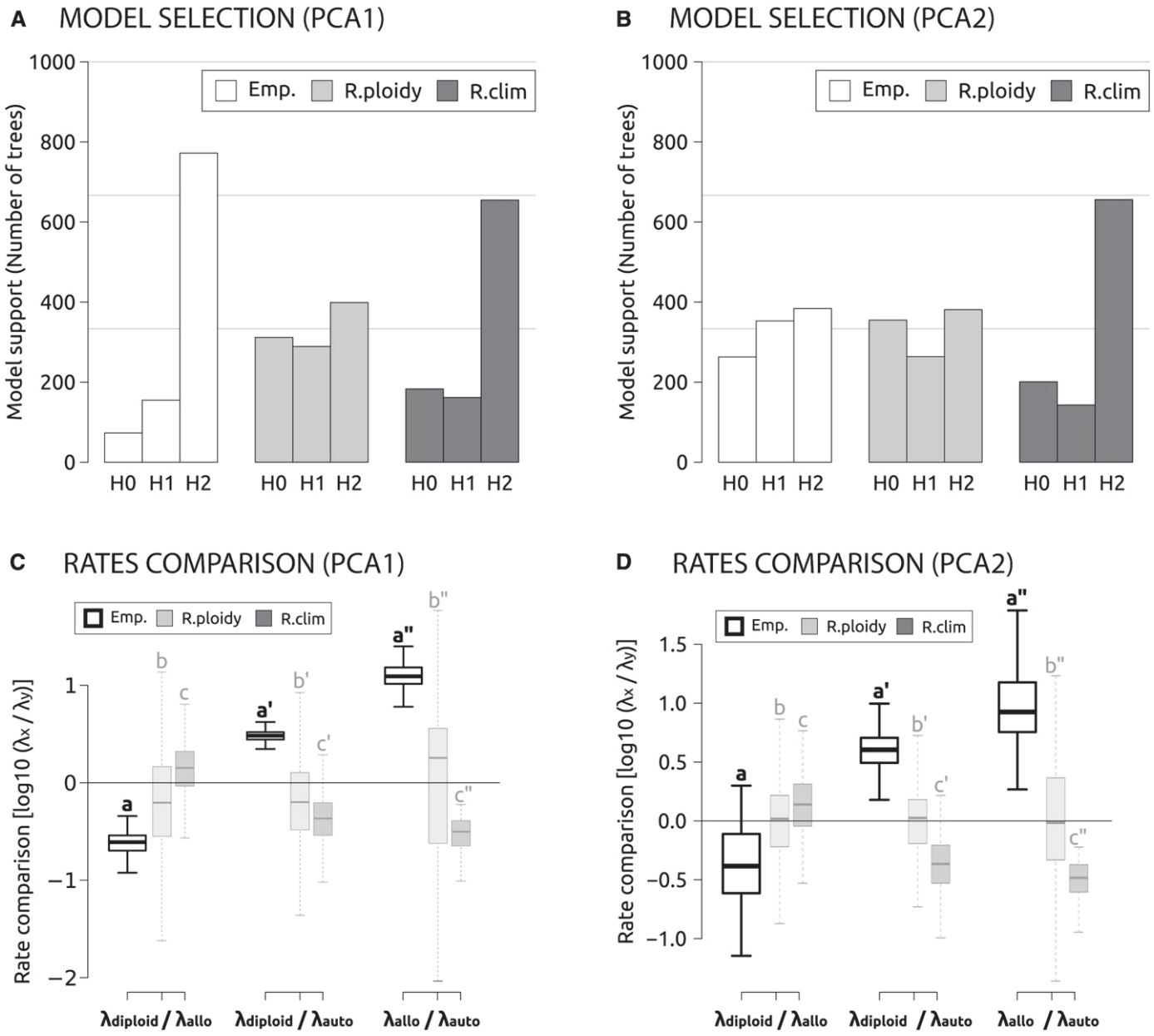

FIGURE 3 Dynamics of climatic niche evolution. We compared the rates of climatic niche evolution estimated for the diploid, allopolyploids, and autopolyploids using phylogeny-based Brownian motion models. We constructed a phylogenetic tree for a subset of 130 populations for which chloroplast DNA sequences were available, using Bayesian inference (see online Appendix S5). We then sampled 1000 trees from the posterior distribution to fit Brownian motion models (see online Appendix S4 for details about the complete procedure). The climatic niche occupied by each of the 130 populations was inferred using the two first eigenaxes (PCA1 and PCA2) of a principal component analysis based on Bioclim variables (see Fig. 2D). For each tree, three models were considered by assuming either (1) a unique rate of climatic evolution for the complete phylogeny (H0), (2) distinct rates for the diploids and polyploids (i.e., allo- and autopolyploids shared the same rate, H1) or (3) distinct rates for the diploids, allopolyploids, and autopolyploids (H2). (A, B) Model selections. The best fitting model of each tree was selected according to the Akaike information criterion (AIC) and the number of trees supporting the H0, H1, and H2 models was displayed here as barplots. (C, D) Parameter estimations. We considered the results of the 1000 Brownie instances and summarized the respective Brownian variances yielded by the diploid, allopolyploids, and autopolyploids using variance ratios, as $\lambda_{diploid} / \lambda_{allo}$, $\lambda_{diploid} / \lambda_{auto}$ and $\lambda_{allo} / \lambda_{auto}$, displayed here as boxplots. Significance levels were assessed using two permutation approaches where either the ploidy levels (R.ploidy) or the PCA1 / PCA2 eigenvalues (R.clim) were reassigned randomly (without replacement) to the phylogeny tips before reapplying the Brownie analysis. The results of those permutations were compared with the empirical results using a nonparametric Kruskal–Wallis test (1000 permutations, applying a Dunn post hoc test and Bonferroni *p*-value adjustment).

increased rates of climatic niche evolution compared with their autopolyploid counterparts. Nevertheless, we outline here at least two limitations that should mitigate our conclusions and foster additional research about the role of hybridization as a driver of ecological novelty. First, climatic data were used as a surrogate of the ecological niche of *Alyssum montanum* s.l., but additional environmental dimensions, such as soil composition and interactions with other species, are also important components of the niche definition. Next, and perhaps more importantly, our study relied on natural populations where the geographical location of allo- and autopolyploid populations was not random. Instead, hybrid zones tend to lock on migration barriers (Barton, 2013; Hewitt, 2011)—corresponding here to mountainous ranges—a feature that may explain why allopolyploids occurred more often in ecologically variable areas. Thus, we cannot strictly exclude that the evolutionary benefits associated with hybridization arise from increased ecological opportunity rather than increased rates of climatic niche evolution. To this respect, controlled hybridization and systematic common-garden experiments may provide a definitive answer (e.g., Hovick and Whitney, 2014).

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