

REVIEW ARTICLE

How much do we know about the frequency of hybridisation and polyploidy in the Mediterranean region?

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Contact zones; ecological differentiation; genome duplications; hybridisation; isolation barriers; Mediterranean Basin; ploidy level; unreduced gametes.

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INTRODUCTION

Throughout the past centuries, interspecific hybridisation and polyploidy have triggered a vast and controversial debate about their importance in species evolution. Although the first ideas argued that hybridisation provided a way for novel species to originate (Lotsy 1916), such ideas were rejected with the emergence of Mayr's concept of species as reproductively isolated populations (Mayr 1940, 1942 and later works). Mayr used and promoted Dobzhansky's (1937) idea of 'isolating mechanisms', which ultimately discouraged many zoologists from accepting interspecific gene flow as an important evolutionary driving force. The rigidity of the biological concept of species caused a delay in acceptance of hybridisation (followed or not by changes in ploidy level) as an important evolutionary mechanism, and even though botanists continued to claim its importance (Anderson & Stebbins 1954; Grant 1981; Abbott 1992; Arnold 1992; Rieseberg 1997; Abbott *et al.* 2013), hybrids and

ABSTRACT

Natural hybridisation and polyploidy are currently recognised as drivers of biodiversity, despite early scepticism about their importance. The Mediterranean region is a biodiversity hotspot where geological and climatic events have created numerous opportunities for speciation through hybridisation and polyploidy. Still, our knowledge on the frequency of these mechanisms in the region is largely limited, despite both phenomena are frequently cited in studies of Mediterranean plants. We reviewed information available from biodiversity and cytogenetic databases to provide the first estimates of hybridisation and polyploidy frequency in the Mediterranean region. We also inspected the most comprehensive modern Mediterranean Flora (*Flora iberica*) to survey the frequency and taxonomic distribution of hybrids and polyploids in Iberian Peninsula. We found that <6% of Mediterranean plants were hybrids, although a higher frequency was estimated for the Iberian Peninsula (13%). Hybrids were concentrated in few families and in even fewer genera. The overall frequency of polyploidy (36.5%) was comparable with previous estimates in other regions; however our estimates increased when analysing the Iberian Peninsula (48.8%). A surprisingly high incidence of species harbouring two or more ploidy levels was also observed (21.7%). A review of the available literature also showed that the ecological factors driving emergence and establishment of new entities are still poorly studied in the Mediterranean flora, although geographic barriers seem to play a major role in polyploid complexes. Finally, this study reveals several gaps and limitations in our current knowledge about the frequency of hybridisation and polyploidy in the Mediterranean region. The obtained estimates might change in the future with the increasing number of studies; still, rather than setting the complete reality, we hope that this work triggers future studies on hybridisation and polyploidy in the Mediterranean region.

polyploids were mostly viewed as 'blind alleys', 'evolutionary dead-ends' or simply 'evolutionary noise' (Stebbins 1950; Wagner 1970; Arrigo & Barker 2012). Still, the idea of hybrids and polyploids as 'monsters' with little evolutionary relevance started to be refuted as some studies continuously revealed the diverse consequences of interspecific gene flow in biodiversity as we know today, including introgression (Rieseberg & Wendel 1993), reinforcement of reproductive barriers (Matute 2010), extinction or displacement of parental species (Rieseberg & Carney 1998) and formation of new stable hybrid lineages through speciation (Rieseberg *et al.* 2003).

Speciation through hybridisation and polyploidy are simplistic designations for complex and long evolutionary processes occurring in nature, where several intermediate stages might occur. Each process itself can occur isolated or combined, and thus hybridisation and polyploidy are extremes of a gradient that largely overlaps (Fig. 1). Hybridisation might occur between species with the same ploidy levels, giving rise to

hybrids with the same number of chromosomes as the parental species (*i.e.* homoploid hybridisation; Fig. 1), but also originating hybrids with chromosomal incompatibilities that are usually sterile due to the impossibility of true pairing at meiosis (Stebbins 1950; Grant 1981). For a long time this process was regarded as evolutionarily irrelevant, since speciation was thought to be unlikely to occur, because without changes in ploidy level and/or in the absence of a physical barrier that limits backcrossing, the hybrid would eventually merge into one or both of the parental species (Levin 1975). Nevertheless, nowadays homoploid hybridisation has been described in a wide variety of organisms, despite it mostly corresponds to diploid hybrids (*e.g.* Buerkle *et al.* 2000; Gross & Rieseberg 2005; Schwenk & Streit 2008; reviewed in Abbott *et al.* 2013; Yakimowski & Rieseberg 2014; Nieto Feliner *et al.* 2017) or stabilised introgressants (*e.g.* Lowe & Abbott 2015). Additionally, in many species, hybridisation is associated with changes in ploidy level, either resulting from hybridisation between heteroploid entities or resulting from genome duplications after homoploid hybridisation (Fig. 1), the latter as a mechanism to stabilise the new hybrid. Interploid crosses were first thought to result in hybrid inviability and sterility (often coined with the triploid block term; Köhler *et al.* 2010), but are now being revealed to be important since they can sometimes result in viable odd-ploidy offspring (Burton & Husband 2000; Husband 2004; Stace *et al.* 2015; Vallejo-Marín *et al.* 2016). Interspecific hybridisation linked with genome duplication events generates allopolyploid entities. Still, new entities can also arise through polyploidy without hybridisation in crosses within or among populations of a single species, producing autopolyploid entities (Clausen *et al.* 1945; Ramsey & Schemske 1998; Fig. 1). This occurs through the fusion of unreduced gametes, directly by the fusion of two unreduced

gametes or indirectly through the formation of odd ploidy offspring that can produce a wide sort of ploidy-variable gametes, working as a triploid bridge (Ramsey & Schemske 1998). Allopolyploids were considered more common in nature than autopolyploids (Grant 1981; Abbott *et al.* 2013), most likely due to difficulties in identifying them, since many autopolyploids remain taxonomically nested within the progenitor species (Soltis *et al.* 2010; Barker *et al.* 2016a). Once again, the boundary between allo- and autopolyploids is too simplistic, as in nature several intermediate situations that promote species divergence might occur (Ramsey & Schemske 1998; Mallet 2007).

Regardless of the routes described above, homoploid hybrids and newly formed polyploids (or neopolyploids) emerge in parental populations, and thus, must overcome competition with parental species to avoid genetic blurring through backcrossing (Coyne & Orr 2004; James & Abbott 2005). Reproductive isolation is, therefore, a key factor in the process of speciation. Under random mating, the establishment of the new entities is subjected to strong frequency-dependent selection (Levin 1975). In early stages, the establishment of new lineages might be favoured by a diverse array of ecological and reproductive features that increase the probability of successful mating (*e.g.* high selfing, asexual reproduction, perenniality, unreduced gamete production; Rausch & Morgan 2005; Rieseberg & Willis 2007; Castro & Loureiro 2014); otherwise, most of the crosses will occur with the progenitors, and the stochastic events originated from the small number of homoploid hybrids and neopolyploids will reduce their chance of establishment (minority cytotype exclusion; Levin 1975; Husband 2000). In later stages, the maintenance of closely related lineages or different cytotypes will only be possible in sympatry if one or more reproductive barriers promote assortative mating;

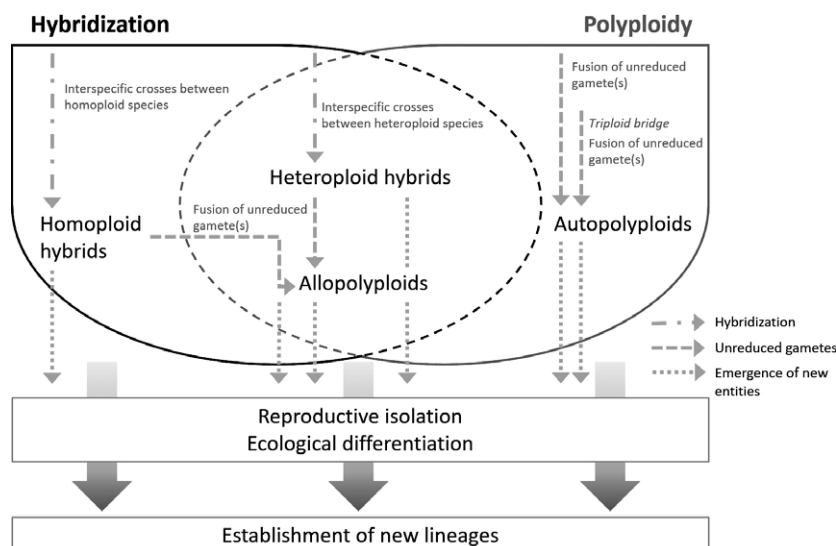


Fig. 1. Schematic representation of the intricate connection between hybridisation and polyploidy in nature. Hybridisation might occur between species with the same ploidy level, giving rise to homoploid hybrids; these might diverge or, because hybrids produce significantly high levels of unreduced gametes, can suffer genome duplications giving rise to allopolyploids. A similar pathway might occur after hybridisation between species with different ploidy levels, giving rise to heteroploid hybrids. The fusion of one or two unreduced gametes in crosses within a species produces autopolyploids, either through an intermediate triploid bridge or directly, respectively. Still, the boundary between allo- and autopolyploids is too simplistic and variable according to the way they are identified. Hybridisation and the fusion of unreduced gametes can lead to the emergence of new entities that might either disappear or become established if a set of isolation barriers and biological attributes promote their success.

otherwise, the new entity would have to disperse to other areas. Indeed, ecological segregation is another factor limiting gene flow, contributing to the process of speciation. The establishment of homoploid hybrids could be facilitated by niche shifts resulting from new tolerances, with the hybrid being able to colonise a new habitat or a new ecological niche (Abbott & Lowe 2004; Marques *et al.* 2016; Vallejo-Marín *et al.* 2016). Similarly, because polyploidy can impact developmental processes, it may lead to shifts in ecological tolerances of the new entities (Levin 2002). Ecological changes linked with increased competitive ability, niche differentiation and/or wider geographic ranges can enhance the ability of new lineages to establish and spread within or beyond their progenitor populations (Levin 2002; Lowry & Lester 2006). In the end, the distribution patterns in nature reflect, among other factors, the dynamics of hybridisation and/or polyploidy events, the nature of the contact zones, the ecological preferences of the different entities and their dispersal abilities, as well as different evolutionary histories (Petit & Thompson 1999; Husband *et al.* 2013).

The Mediterranean region: a hotspot for hybridisation and polyploidy?

The use of molecular tools revolutionised the study of hybridisation and polyploidy, revealing many unknown cases of plant lineages, as well as animals, where these processes have facilitated speciation (Arnold 1992; Dowling & Secor 1997; Seehausen 2004; Grant *et al.* 2005; Soltis *et al.* 2009, 2016; Wendel 2015). On average, it has been estimated that around 25% of plant species and 10% of animal species hybridise naturally (Mallet 2007). Recent population studies of cytogenetic diversity also revealed numerous examples of species embracing several ploidy levels in current natural populations, with 17% of fern species and 12–13% of angiosperm species being estimated to be ploidy-variable (Wood *et al.* 2009; Husband *et al.* 2013). Additionally, cytological, fossil and genomic studies suggested that 47% up to 100% of angiosperms could be traced to a polyploid event at some point of their evolutionary history (Grant 1981; Masterson 1994; Cui *et al.* 2006; Soltis *et al.* 2009; Amborella Genome Project 2013; Wendel 2015). Wood *et al.* (2009) estimated that 31% of fern and 15% of angiosperm speciation events were accompanied by ploidy level increases. These frequencies lead to a standing incidence of polyploid species of 35% (Wood *et al.* 2009), a value similar to those previously obtained by Stebbins (1938, 1950; 20–40%). But how frequent is hybridisation and polyploidy in the Mediterranean region?

Over 25,000 species of vascular plant occur in the Mediterranean region, which is a very high number in comparison with the 6000 plant species recorded in Europe outside the Mediterranean Basin, despite of the latter being represented by an area three to four times larger in size than the Mediterranean region (Quézel 1999). The geological and historical climatic context of the Mediterranean region suggests that there may have been numerous opportunities for the occurrence of hybridisation and polyploidy. First, the successive changes in land connections during different geological events and/or the evolution and oscillation of climate regimes have led to significant changes in species range distribution, including reduction and isolation of populations and subsequent expansion. This has brought into contact previously isolated lineages from different

biogeographic areas and, thus, created opportunities for hybridisation (Hewitt 2000; Thompson 2005; Nieto Feliner 2014). Human activities have also played a major role in the formation of Mediterranean landscapes, resulting in a mosaic of further opportunities for contact between previously isolated species (Quézel *et al.* 1990). Second, environmental stress mediated by the climatic changes recorded in the Mediterranean could have potentiated the emergence of new entities through increased production of unreduced gametes, a fundamental step for polyploid emergence and hybrid stabilisation (Ramsey & Schemske 1998; Brownfield & Köhler 2011; Mason & Pires 2015). Several studies have shown a relation between the production of unreduced gametes and environmental stress (reviewed in Ramsey & Schemske 1998), particularly in response to changes in temperature. For example, unreduced gamete production increased significantly after temperature fluctuations (including both cold and heat conditions; *e.g.* Mason *et al.* 2011; Pecrix *et al.* 2011; De Storme *et al.* 2012). This suggests that natural environmental changes (*e.g.* altitudinal gradients), as well as large-scale climate changes (as shown in Kürschner *et al.* 2013; Vanneste *et al.* 2014) could substantially alter the dynamics of polyploid evolution, or at least fuel opportunities for establishment through the recurrent formation of new entities. Finally, hybridisation and genome duplications have been related to the emergence of novel traits that might facilitate the colonisation of novel habitats and/or range expansion, or different abilities to cope with climate changes (Levin 2002; Trewick *et al.* 2002; Maherali *et al.* 2009; Balao *et al.* 2010; Manzaneda *et al.* 2012, 2015; Hao *et al.* 2013; Vamossi & McEwen 2013; Marques *et al.* 2016; Vallejo-Marín *et al.* 2016).

Altogether, hybridisation and polyploidy are expected to be fairly common in the Mediterranean flora and probably important motors of diversification in this region. It is therefore not a surprise to see these processes frequently cited in several plant studies focused on Mediterranean groups (*e.g.* *Armeria* Willd., *Tauleigne-Gomes* & *Lefebvre* 2005; *Narcissus* L., Santos-Gally *et al.* 2012; *Orchis* L., Cozzolino *et al.* 2006; *Phlomis* L., Albaladejo & Aparicio 2007; *Centaurium* Hill, Mansion *et al.* 2005; among many others). Still, to date, there are no estimations of the frequency of hybridisation and polyploidisation in the Mediterranean region, and accessing them is particularly challenging given the diversity of the Mediterranean at several levels.

In this review, we provide first estimates on the frequency of hybridisation and polyploidy in the Mediterranean region, by combining information from the botanical community's vast biodiversity and cytogenetic databases for the Mediterranean area. The few available ecological studies exploring the conditions involved with establishment and spread of new lineages in Mediterranean plant complexes are also briefly presented. The search included ferns and allies, conifers and flowering plants with geographic coordinates in the 3,500,000 km² Mediterranean biogeographic area that stretches across three continents (Europe, Africa and Asia) and 24 different countries following the geographic boundaries of the Mediterranean region defined in Blondel *et al.* (2010). We realise that this approach limits our estimations to information that is readily accessible in databases, but compiling it using the vast and fragmented scientific literature published so far seems unrealistic. Also, modern Floras with the information necessary to

conduct our survey are limited. For instance, hybrids are not reported in most cases (e.g. Flora of Italy, Pignatti 1982) and when listed, there is frequently no description of the hybrid and of the parent species (but see Castroviejo 1986–2014 for an exception). In addition, different taxonomic approaches have been adopted in the available Floras, including the use of extremely synthetic criteria [e.g. criteria followed by Tutin *et al.* (1964–1993) in Flora Europaea], which were subsequently followed in the Floras of specific territories (e.g. Portugal: Franco 1971–1984; Franco & Afonso 1994, 1998, 2003), ultimately leading to a scarcity of hybrid reports. Also, although chromosome counts have long been used by botanists as an important taxonomic tool, the information is still limited for a high number of plant species [e.g. Bennett (1998) estimated that chromosome numbers have been determined for only about 25% of angiosperm species], a fact that is aggravated by different taxonomic treatments across local Floras and by the use of one or few individuals to assign the ploidy level of a given species, without exploring the further diversity that might occur across its distribution range. The information on chromosome counts is also largely scattered in numerous publications across botanical journals, being extremely difficult to compile. Thus, quantifying hybrids and polyploids based on comprehensive databases enable us, to some degree, to overcome these limitations, while providing global estimations of their frequency for the Mediterranean region. Being aware of the potential limitations of this wider approach, we have made additional analyses focused on a particular region within the Mediterranean (Iberian Peninsula) using the *Flora iberica* (Castroviejo 1986–2014). *Flora iberica* is one of the best-known Floras in the Mediterranean region, being almost complete (only Asteraceae and Poaceae remain to be published), although it also includes other biogeographic regions (not considered in the previous approach). This Flora provides a list of hybrids and generally their parental species, and revises all available chromosome counts. But it is also an exception within the Mediterranean region, precluding general conclusions due to limitations in space and time (Yakimowski & Rieseberg 2014), and the fact that the information on hybrids and polyploids varies with the systematic attention given to a specific family or genus. The quantifications provided here should be regarded as baseline numbers, and thus, the frequency of hybridisation and polyploidy might be different when the number of studies is increased. Nevertheless, rather than setting the complete reality, we expect that the quantifications presented here constitute a road map to new and inspiring studies in the Mediterranean region.

HYBRIDISATION AND POLYPLOIDY IN THE MEDITERRANEAN REGION

Distribution and frequency of hybrids

To estimate the distribution and frequency of hybrids in the Mediterranean region, we searched for all known vascular plant species (17,020 genera and 350,699 species) accepted by the most comprehensive working list of all known plants (The Plant List 2013) and counted all the hybrids formally described as such (e.g. with the hybrid multiplication sign \times) or any *notho* rank in each accepted family and genus. The Global Biodiversity Infrastructure Facility (GBIF) Backbone Taxonomy

was adopted to solve the taxonomic problems (e.g. synonyms). In the case of intergeneric hybrids, they were only counted once, and the hybrids were attributed to the first genus listed. We narrowed our search to specimens with geographic coordinates in the Mediterranean bioregion (following Blondel *et al.* 2010), as besides being our study area, we also wanted to evaluate if hybrids were being cited more frequently in some specific territory. We based our search on the GBIF using the “dismo” package in R software (Hijmans & Elith 2013). This allowed us to have a broader picture of hybridisation across plant lineages, enabling, at the same time, identification of the genera where hybridisation is more frequent. However, we should point out that although the Plant List constitutes the largest database assembled so far, covering the entire world, and many Floras, it is far from being complete. Furthermore, there is an unbalanced data contribution to GBIF, with the biodiversity being better known in some countries than in others, thus resulting in an underrepresentation of certain territories in our dataset. The search for hybrid plants in databases is also very complex. For instance, our search in GBIF excluded the hybrid species where the specific epithet (hybrid multiplication sign \times or *notho* epithet) has been omitted from its name, or even hybrids where ploidy changes have occurred and a new nomenclatural name was given, since these entities were not traceable during the search. Finally, *The International Code of Nomenclature for Algae, Fungi, and Plants* does not require that a name should be given for plants believed to have a hybrid origin (McNeill *et al.* 2012); still, when formally described, hybrid plants should appear in our search. The classification of the families followed Brummitt (1992).

In total, we searched for 23,675 taxa: 320 ferns and allies, 179 gymnosperms and 23,176 angiosperms with geographic coordinates in the Mediterranean region (GBIF.org 2017). From this list, we found that 5.6% of the plants were hybrids (representing 1323 hybrids among the total number of 23,675 specimens recorded). This average is similar to estimations found by Ellstrand *et al.* (1996) based on local Floras from the Intermountain West (5.4%) or from the Great Plains (5.7%), but considerably smaller than that obtained in extensively studied areas like the British Isles (17.8%; Table 1). The number of hybrids recorded in the Mediterranean region also varied considerably between lineages: hybrids were frequent in ferns and allies, with 14.4% of all taxa from this group being hybrids,

Table 1. Comparison of the frequency of reported hybrids estimated for different biogeographic regions (white rows), including the Mediterranean biogeographic region (Mediterranean) and the Iberian Peninsula analyzed in this study (grey rows). The values for the other regions were calculated from Ellstrand *et al.* (1996), who based their estimations on five biosystematic Floras from Europe (British Isles and Scandinavia), North America (Great Plains and the Intermountain West) and one tropical region (Hawaii).

region	frequency of hybrids (%)	families (%)	genera (%)
British Isles	17.8	34	16
Scandinavia	8.7	31	14
Great Plains	5.7	21	8
Intermountain	5.4	31	12
Hawaii	8.5	16	6
Mediterranean	5.6	21	8
Iberian Peninsula	12.7	32	14

while only 2.8% of all gymnosperms and 5.5% of all angiosperms recorded in the Mediterranean were found to be hybrids.

A clear conclusion from our approach was the existence of huge gaps in information regarding hybrid occurrence in the Mediterranean area. Such discrepancies in information are easily visible in Fig. 2 where a large number of plant hybrids has been recorded in some countries of the western Mediterranean Basin (e.g. Spain, France); in contrast, only a few available records were found in the eastern Mediterranean area and along the whole North African coast, despite such low numbers being hardly realistic. This is however a reality for all species (not only for hybrids), since the eastern Mediterranean region is underrepresented in the GBIF database (<http://www.gbif.org/occurrence> last accessed 13-03-2017). At the other extreme, it is important to highlight the presence of several 'hotspots of hybrid records' around the confluences of the Mediterranean, Atlantic and Alpine biogeographic regions (Fig. 2), which is not surprising given its historical features. Indeed, the spatially heterogeneous climate of that area (Thompson 2005) might have provided conditions for recurrent hybridisation events. However, it is important to highlight that these quantifications only give us an estimate of hybrid records, not necessarily implying new stabilised hybrid lineages. How many of these records represent single individuals and how many are or will be in the process of speciation is something that will remain unsolved.

Despite the paucity of available information and the biases while searching the GBIF database, our search allowed identifying certain trends in the Mediterranean region. As expected, hybrids were not widespread or evenly distributed taxonomically, but were rather concentrated in some families and genera: 59 families and 165 genera had at least one hybrid recorded in the Mediterranean biogeographic region, resulting in the representation of hybrids in 21% of families and 8% of genera, only. These estimates are similar to those reported for other biogeographic regions, with the exception of territories where hybrids have been traditionally recorded, such as the British Isles (Table 1). Figure 3 shows the top ten families and genera with the higher frequency of hybrids considering the total number of taxa recorded in those groups. It is not

surprising to see that many of the groups where hybrids were frequent are primarily perennials or can spread through asexual reproduction, since these mechanisms may increase the chances of establishment of newly formed hybrids (Ellstrand *et al.* 1996; Fig. 3). However, to have a better picture of the frequency of hybrids in the Mediterranean region, it is crucial to improve and increase the available information without neglecting the hybrid records.

Additionally, similarly to Ellstrand *et al.* (1996), we wanted to evaluate if the Mediterranean patterns described above were maintained using a regional approach based on a modern Flora. For that, we counted all the hybrids included in all genera and families represented in the Iberian Peninsula, as listed in the *Flora iberica* (Castroviejo 1986–2014). We also explored the representation of Iberian hybrids in public databases, and if they are well georeferenced. For that, we searched all the hybrids (as well as their synonyms) recorded in *Flora iberica* on GBIF using the "dismo" package in R software (Hijmans & Elith 2013), restricting the search to Iberian Peninsula records. The Basic Herbaria supporting *Flora iberica* are data providers of the GBIF consortium (but only 58.9% of their collections are available online; GBIF.ES Nodo Español de GBIF 2015; CJBG, Conservatoire et Jardin botaniques de la Ville de Genève 2017), except the Herbarium of the University of Coimbra (COI). To cover the COI Herbarium, the list of hybrids (including synonyms) was searched directly in the COI online catalogue (<http://coicatalogue.uc.pt/>; last accessed 10-03-2017). Further searches of hybrids in specific regions of the Iberian Peninsula were not done, due to the low number of hybrids listed in regional Floras. The families were classified following *Flora iberica* (Castroviejo 1986–2014).

Based on this approach, we found 1,032 hybrids currently accepted in *Flora iberica* (and 1,446 synonyms related to those accepted hybrids), which would indicate that approximately 13% of all taxa reported for the Iberian Peninsula are hybrids (Table 1). From these, only 11% of Iberian hybrids have any record available in public databases (113 accepted hybrids found in 1,955 records). These numbers clearly show the gap of information on hybrids and their distribution, although it is possible that several hybrids are still pending computerisation.

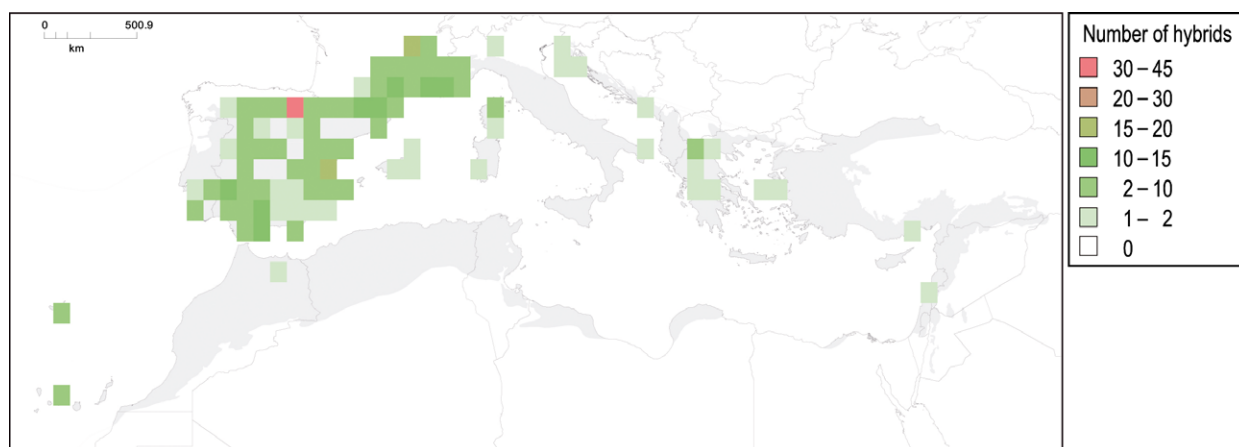


Fig. 2. Number of plant hybrids with geographic coordinates in the Mediterranean biogeographic region (grey), that comprises approximately 5300 km west–east and 2200 km north–south across three continents (Europe, Africa and Asia) and 24 different countries (following Blondel *et al.* 2010). Pixel resolution: 1 × 1 degree. Colour legend indicates the number of hybrids recorded. An absence of colours indicates that no information was available for that pixel.

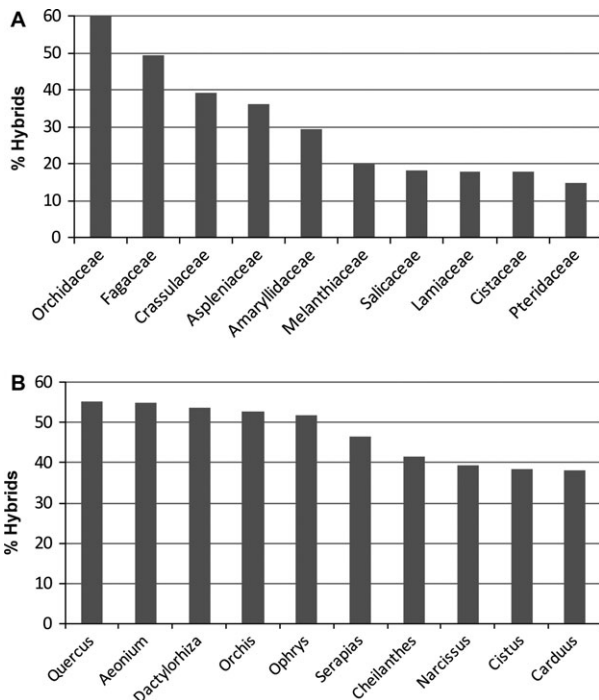


Fig. 3. Top ten families (A) and genera (B) with the highest frequency of hybrids considering all the species recorded in the Mediterranean area.

As expected, and as observed for the Mediterranean region, hybrids in the Iberian Peninsula were also taxonomically restricted, being concentrated in 32% of the families and 14% of the genera, only (Table 1). Hybrids were only found in 57 families, and of these, four families accounted for almost half of all hybrids described in the Iberian Peninsula, namely, Plumbaginaceae (179 hybrids), Labiatae (171 hybrids), Orchidaceae (91 hybrids) and Rosaceae (76 hybrids), although these families were also among those with a higher number of synonyms (Table S1). However, when we take into consideration family size (*i.e.* total number of taxa accepted), ten families appeared as important hybridising families in the Iberian Peninsula: Fagaceae, Salicaceae, Amaryllidaceae, Plumbaginaceae, Violaceae, Aspleniaceae, Orchidaceae, Sinopteridaceae, Polypodiaceae and Typhaceae (Table 2). In those families, more than half of the *Flora iberica* reported taxa are hybrids. Most of these hybrids belong to monocot families, but they are also well represented in Aspleniaceae, Sinopteridaceae and Polypodiaceae, important families of the Pteridophytes (Table 2). The frequency of hybrids drops considerably for the remaining Iberian families; in fact, most of remaining families (31 families out of 57) have a small number of recorded hybrids, accounting for less than 25% of the reported taxa in each of those families (Table S1).

In the Iberian Peninsula, 121 out of 845 published genera (14.3%) had at least one hybrid reported. Figure 4 shows the top ten genera with the highest number of hybrids in the Iberian Peninsula (see Table S2 for complete information), revealing a remarkable number of hybrids in the genus *Limonium* Mill., followed by *Thymus* L., *Rosa* L. and *Narcissus*. In accordance, several studies have documented the importance of hybridisation in the evolution of the genera highlighted in Fig. 4 (*e.g.* *Limonium*: Lledó *et al.* 2005; Castro & Rosselló

2007; *Thymus*: López-Pujol *et al.* 2004; *Armeria*: Piñeiro *et al.* 2011; *Ophrys* L.: Cotrim *et al.* 2016; *Narcissus*: Marques *et al.* 2010). Remarkably, those genera also present taxonomic problems, with species boundaries being sometimes difficult to define (*e.g.* *Limonium*: Palacios *et al.* 2000; *Armeria*: Fuertes Aguilar *et al.* 1999); in fact, a high number of synonyms were found in *Flora iberica* for those genera (Table S2). However, if we take into account the size of the genus, our data revealed that hybridization was frequent only in 23% of the genera (28 out of 121 genera presented a hybrid frequency higher than half of all the taxa described for a given genus; Table 3). The frequency of hybridisation dropped considerably for the remaining genera, and more than one third present a very low frequency of hybrids (45 genera out of 121 have a hybrid frequency <25%; Table S2). It should also be highlighted that a high number of genera (85.7%, 726 from 845) have no single hybrid reported in the Iberian Peninsula. This does not necessarily mean that hybridisation is absent from those genera; nevertheless, it is evident that hybrids are absent from a high number of well-studied families, including some that are relatively large.

Finally, the families and genera that seemed to be prone to hybridisation varied between the two approaches, and a substantially higher number of hybrids were found in the Iberian Peninsula. This trend was expected, as the Iberian Peninsula is a well-studied region with a vast number of taxonomic studies being performed in this region in the last decades (Castroviejo 1986–2014). The complex geology, diverse substrates and climate of the Iberian Peninsula (Thompson 2005) have certainly acted as a melting point for multiple scenarios of plant diversification, including hybridisation events. Still, estimations based on a Flora approach have certain biases, since the report of hybrids may vary among taxonomic groups and according to the systematic criterion given (*e.g.* from more narrow approaches to lumping classifications). An example of that is the high number of synonyms found in certain families and genera (Tables 1 and S1). Furthermore, we found a low number of hybrids available in public databases. Some vouchers of hybrid plants are still in personal collections without representatives in any herbarium and are therefore difficult to access. Also, because the importance of hybrids has for long been dismissed, hybrid vouchers are usually maintained separately and left untreated (including without computerisation) until detailed taxonomic revisions. The heterogeneity in the different formulas used to describe hybrids accepted by the Melbourne Code (<http://www.iapt-taxon.org/nomen/main.php?page=title>) also complicates the computerisation process itself. In addition, the standards used in the computerisation process of biological collections are not prepared to incorporate and use data from hybrid plants; for example, in GBIF it is not possible to perform searches for the “hybrid” attribute unless they include the multiplication sign (×) or any *notho* rank (F. Pando, personal communication).

Frequency of polyploids

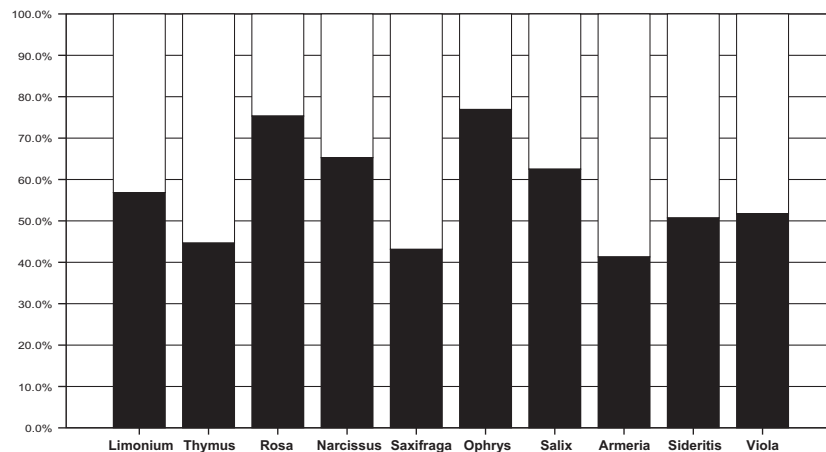
To estimate the incidence of polyploid species in the Mediterranean region, we followed the classical approach used by Stebbins (1938) with modifications, *i.e.* the estimates were based on the proportion of species that have chromosome numbers that are more than double of the base number of the genus.

Table 2. Top ten families with the highest frequency of hybrids recorded in the Iberian Peninsula according to *Flora iberica* (Castroviejo 1986–2014). The families are ranked from the highest to the lowest hybrid frequency (% of hybrids according to the total number of taxa accepted for the family). The number of species currently accepted in *Flora iberica*, excluding hybrids (No. of species), the number of synonyms of the hybrids accepted by *Flora iberica* (No. of synonyms of the hybrids), the number of accepted hybrids available in public databases (No. of hybrids available in public databases; GBIF and the COI Catalogue: <http://coicatalogue.uc.pt/>; last accessed 10-03-2017), and the number of records available and the number of georeferenced records both provided by *Flora iberica* and from other sources included in GBIF, including observations (No. of records available and No. of georeferenced records, respectively), are also provided.

family	no. of species	no. of hybrids	frequency of hybrids (%)	no. of synonyms of the hybrids	no. of hybrids available in public databases	no. of records available	no. of georeferenced records
Fagaceae	14	23	62.2	50	10	445	358
Salicaceae	26	40	60.6	59	7	313	232
<i>Amaryllidaceae</i>	34	47	58.0	172	12	51	24
Plumbaginaceae	164	179	52.2	36	4	492	290
Violaceae	28	30	51.7	61	0	0	0
Aspleniaceae	19	20	51.3	22	0	0	0
<i>Orchidaceae</i>	90	91	50.3	180	7	14	10
Sinopteridaceae	7	7	50.0	7	0	0	0
Polypodiaceae	3	3	50.0	4	0	0	0
<i>Typhaceae</i>	3	3	50.0	3	1	6	6
Pteridophytes	56	42	41.1	49	2	3	0
Gymnosperms	13	1	7.1	1	0	0	0
Angiosperms	3978	989	22.3	1396	111	1954	1168
Dicots	3437	796	20.4	1003	82	1845	1114
Monocots	541	193	29.1	393	29	109	54
Total	4047	1032	24.1	1446	113	1957	1168

Light grey rows indicate Pteridophyte families. Within angiosperms, monocot families are indicated in italics. A summary for the major plant groups (intermediate grey) and for the whole dataset (dark grey) is also given, indicating total numbers (including only the families where at least one hybrid has been reported), or the average in the case of the frequency of hybrids.

Fig. 4. Top ten Iberian genera ordered by the highest numbers of hybrids recorded. Black bars represent the proportion (in percentage, %) of hybrids accepted in *Flora iberica* with respect to the total number (grey bars) of taxa recognised in the Iberian Peninsula for a particular genus.



Whenever doubts arise, we searched in the literature for the base chromosome number, x , of particular genera and/or species. A conservative and conventional approach was taken, and polyploids were only considered when evidence for more than one ploidy level was observed, and thus cryptic evidence of polyploidy, such as multiple copies of whole genome sequences or genome segments, or diploidisation events were not considered. Consequently, this method enabled us to depict recent polyploidy events, since it was unrealistic to consider ancient ones at the scale of this study. Finally, the available information on the origin of polyploids is very scarce and, thus, it was impossible to decouple the frequency of auto- versus allopolyploidy.

Three geographic approaches were followed, each referring to different scientific literature. First, all the Mediterranean chromosome number reports published in *Flora Mediterranea* were gathered from the Chromosome Counts Database (CCDB; Rice *et al.* 2015; <http://ccdb.tau.ac.il/>, last accessed 01-03-2017) and used to compile the information available for the Mediterranean region in an integrated view of the entire region (1st approach). Second, and following a similar approach as above for the hybrids, we focused on the Iberian Peninsula (2nd approach). For that, all the published volumes of *Flora iberica* (Castroviejo 1986–2014) were analysed, and the following data were extracted for each species: absence/presence of information about the chromosome number (as 0/1,

Table 3. Top genera growing in the Iberian Peninsula where the frequency of hybrid plants is more than half of the total number of taxa described for that genus following *Flora iberica* (Castroviejo 1986–2014). The genera are ranked from the highest to the lowest hybrid frequency (% of hybrids according to the total number of taxa accepted for the genus). The number of species currently accepted in *Flora iberica*, excluding hybrids (No. of species), the number of synonyms of the hybrids accepted by *Flora iberica* (No. of synonyms of the hybrids), the number of accepted hybrids available in public databases (No. of hybrids available in public databases; GBIF and the COI Catalogue: <http://coicatalogue.uc.pt/>; last accessed 10-03-2017), and the number of records available and the number of georeferenced records both provided by *Flora iberica* and from other sources included in GBIF, including observations (No. of records available and No. of georeferenced records, respectively), are also provided.

family	genus	no. of species	no. of hybrids	frequency of hybrids (%)	no. of synonyms of the hybrids	no. of hybrids available in public databases	no. of records available	no. of georeferenced records
Orchidaceae	<i>Ophrys</i>	12	40	76.9	91	6	9	6
Rosaceae	<i>Rosa</i>	19	58	75.3	51	3	6	0
Orchidaceae	<i>Aceras</i>	1	3	75.0	11	0	0	0
Orchidaceae	<i>Dactylorhiza</i>	8	18	69.2	39	1	5	4
Orchidaceae	<i>Anacamptis</i>	1	2	66.7	2	0	0	0
Amaryllidaceae	<i>Narcissus</i>	25	47	65.3	172	12	51	24
Fagaceae	<i>Quercus</i>	12	22	64.7	49	10	445	358
Salicaceae	<i>Salix</i>	24	40	62.5	59	7	313	232
Cistaceae	<i>Cistus</i>	12	20	62.5	77	7	31	6
Orchidaceae	<i>Gymnadenia</i>	2	3	60.0	8	0	0	0
Aspleniaceae	<i>Asplenium</i>	15	20	57.1	22	0	0	0
Plumbaginaceae	<i>Limonium</i>	107	141	56.9	26	3	491	290
Labiatae	<i>Prunella</i>	4	5	55.6	13	1	5	5
Sinopteridaceae	<i>Cheilanthes</i>	6	7	53.8	7	0	0	0
Labiatae	<i>Lavandula</i>	8	9	52.9	14	2	7	4
Violaceae	<i>Viola</i>	28	30	51.7	61	0	0	0
Polygonaceae	<i>Rumex</i>	25	26	51.0	24	2	5	4
Cistaceae	<i>Helianthemum</i>	24	25	51.0	49	2	57	5
Labiatae	<i>Sideritis</i>	34	35	50.7	51	0	0	0
Labiatae	<i>Mentha</i>	8	8	50.0	14	1	33	6
Euphorbiaceae	<i>Mercurialis</i>	7	7	50.0	6	1	1	0
Rosaceae	<i>Geum</i>	7	7	50.0	15	0	0	0
Iridaceae	<i>Romulea</i>	4	4	50.0	11	0	0	0
Polypodiaceae	<i>Polypodium</i>	3	3	50.0	4	0	0	0
Typhaceae	<i>Typha</i>	3	3	50.0	3	1	6	6
Geraniaceae	<i>Pelargonium</i>	2	2	50.0	4	0	0	0
Orchidaceae	<i>Himantoglossum</i>	1	1	50.0	3	0	0	0
Orchidaceae	<i>Barlia</i>	1	1	50.0	1	0	0	0
Pteridophytes		52	42	40.2	49	2	3	0
Gymnosperms		7	1	12.5	1	0	0	0
Angiosperms		1780	989	31.23	1396	111	1954	1168
	Dicots	1480	796	28.4	1003	82	1845	1114
	Monocots	300	193	40.9	393	29	109	54
Total		1839	1032	31.6	1446	113	1957	1839

Light grey rows indicate Pteridophyte genera. Within angiosperms, monocot families are indicated in italics. A summary for the major plant groups (intermediate grey) and for the whole dataset (dark grey) is also given, indicating total numbers (including only the families where at least one hybrid has been reported), or the average in the case of the frequency of hybrids.

respectively), state diploid/polyploid (as 0/1, respectively), and absence/presence of more than one ploidy level (as 0/1, respectively). Exotic species were excluded from the dataset. Because Asteraceae and Poaceae have not yet been published in *Flora iberica*, data on these families were gathered from available databases of the Iberian Peninsula flora, namely Anthos (2016; <http://www.anthos.es/>, last accessed 15-02-2017) and CROMOCAT (Simon & Blanché 2016; <http://biodiver.bio.ub.es/biocat/>, last accessed 15-02-2017). Finally, because Iberian Peninsula includes other biogeographic regions, a subset of the compiled data from the Iberian Peninsula was extracted (3rd approach), using the taxa reported to occur in the Andalusia autonomous community (approximately 15% of the Iberian Peninsula area),

a typical Mediterranean region with available Floras (Valdés *et al.* 1987; Blanca *et al.* 2011). Classification of the families followed Brummitt (1992) for the 1st approach, and *Flora iberica* (Castroviejo 1986–2014) for the 2nd and 3rd approaches.

The following plant groups were considered: Pteridophytes, Gymnosperms and Angiosperms (divided also in Monocots and Dicots *s.l.*). For each group and geographic approach, the following parameters were calculated: percentage of species with chromosome number information (not applicable for the Mediterranean region), percentage of species with polyploids, percentage of species with more than two ploidy levels. Within each plant group, the same approach was followed at the family level. A geographic approach, as that made for hybrids, was not

feasible since Floras and herbarium vouchers rarely provide geographic information associated with chromosome numbers. The distribution patterns of cytotypes are only available from detailed studies on particular plant groups, and to consider them within the scope of this study was considered unrealistic.

We realise that the search for information was not fully exhaustive outside the Iberian Peninsula. Similar to the study of hybrids, fragmentation of the information available, together with the use of different taxonomic treatments and ploidy levels based on chromosome counts of one or few individuals without clear knowledge from the entire distributional ranges, made it difficult to compile information from an extensive area such as the Mediterranean region. Thus, the data gathered in the 1st approach certainly require further updates. Nevertheless, overall, it was possible to compile data from 693 Mediterranean species (1st approach), 5,974 Iberian Peninsula species of which 4,821 had information (2nd approach), and 3,448 Andalusian species of which 2,896 presented information (3rd approach). Considering that previous estimates suggested that chromosome numbers had been determined for about 25% of angiosperm species (Bennett 1998), it was a surprise to discover that over 80% of the Iberian taxa has at least one chromosome count.

Total estimates of polyploid frequency in the Mediterranean region ranged from 36.5% (1st approach) to 48.8% (2nd approach) (Tables 4 and S3, Fig. 5). In the particular case of the Iberian Peninsula dataset, the frequency of polyploids was

Table 4. Angiosperm families for which information on chromosome numbers and/or ploidy level(s) is available for 20 or more species occurring in the Mediterranean region. The families are ranked from the highest to the lowest polyploid frequency.

family	number of taxa	polyploidy frequency (%)	mixed-ploidy species within polyploids (%)	mixed-ploidy species from total (%)
Orchidaceae	21	95.2	0.0	0.0
Lamiaceae	48	54.2	19.2	10.4
Poaceae	26	46.2	8.3	3.8
Brassicaceae	32	43.8	14.3	6.3
Boraginaceae	28	39.3	0.0	0.0
Caryophyllaceae	65	38.5	4.0	1.5
Fabaceae	155	25.2	2.6	0.6
Chenopodiaceae	26	23.1	16.7	3.8
Asteraceae	48	20.8	0.0	0.0
Apiaceae	42	2.4	0.0	0.0
Pteridophytes	4	75.0	0.0	0.0
Gymnosperms	5	80.0	0.0	0.0
Angiosperms	684	36.0	7.3	2.6
Dicots	577	32.1	7.0	2.3
Monocots	107	57.0	8.2	4.7
Total	693	36.5	7.1	2.6

White rows represent dicot families, while light grey rows refer to monocot families. A summary for the major plant groups (intermediate grey) and for the whole dataset (dark grey) is also given. The number of species for which there was available information, the percentage of polyploid species (Polyploidy frequency), and the percentage of mixed-ploidy species with respect to the total number of polyploid species (Mixed-ploidy species within polyploids) or to the total number of species (Mixed-ploidy species) are provided for each family.

higher for the Pteridophytes (74.5%), followed by the Angiosperms (48.3%), while the Gymnosperms presented a much lower frequency (6.7%) (Table S3, Fig. 5B). Similar values were obtained for the Andalusian subset (Table S4, Fig. 5C). Unfortunately, in the 1st approach information available for the Pteridophytes and Gymnosperms was extremely scarce (Table 4).

The differences in polyploid frequency among the three major plant groups evaluated are in accordance with the literature. Pteridophytes that include ferns and allies (Monilophytes) and lycopods are considered the plant group with the highest frequency of polyploidy among all plants (Barker 2013). According to Grant (1981), 95% of the monilophytes have suffered one or more rounds of genome duplication during their evolutionary history. Still, more conservative approaches, that include the possibility of chromosomal diploidisation events during the evolution of this lineage, suggested that only 43.5% of ferns and allies are polyploid (Vida 1976). In our study, fairly high values were obtained independently of the geographic approach (>74%). Additionally, in 31.6% and 28.6% of the cases (Fig. 5B, C for Iberian Peninsula and Andalusia, respectively), mixed-ploidy taxa were observed, which is a value slightly higher than that obtained by Wood *et al.* (2009) for fern species (17%).

With respect to Gymnosperms, a group very well studied from a karyological point of view (Murray 2013), chromosome number information was available for 55.6% of the species of the Iberian Peninsula, only. Of these, only a very small fraction (6.7%) was polyploid. This was expected, as the extant gymnosperms constitute the plant group with less evidence of polyploidy events (Murray 2013). Usually, taxa belonging to gymnosperms already present large monoploid genome sizes (Husband *et al.* 2013); therefore, if whole genome duplications would occur, the subsequently higher genome sizes could pose a constraint to plant development, as observed in sporadic polyploids and aneuploids obtained in nurseries of conifers, which showed growth abnormalities, dwarfism and most did not reach maturity (Ahuja 2005).

Angiosperms constitute the plant group for which there is more information about karyological counts (although not proportionally). Here, we obtained estimations of polyploidy frequency varying between 36.0% and 48.3% (Tables 4, S3 and S4). Previous estimations are largely variable (30–80%) and dependent of the method used to calculate polyploid frequency (Otto & Whitton 2000; Husband *et al.* 2013). Our estimations for the Mediterranean (36.0%) fall within the expected numbers, being similar to recent estimates for Mediterranean regions from the northern hemisphere (30–40%; Oberlander *et al.* 2016) and to other overall estimates (35% for vascular plants; Wood *et al.* 2009). However, when we studied the floras of particular regions of the Mediterranean in detail, the polyploid frequency increased, with Iberian Peninsula and Andalusia presenting higher values (48.3% and 47.5%, respectively) than previous estimates. Similar values were observed in other regions of the world, such as for example, the flora from British Columbia, Canada, where 45.7% of the species were polyploids, including 12.3% of polyploids of hybrid origin (Vamosi & McEwen 2013). These allopolyploids presented an increased elevational range, suggesting that the production of novel phenotypes, as well as a wider range of allelic diversity, are important factors determining the range limits of a species (Vamosi

& McEwen 2013). Interestingly, species richness in northwest USA and Canada has been related to climate change events and barriers to species dispersal generated during glaciation periods (Gavin 2009). The circumarctic flora also revealed a high frequency of polyploids, with 60.7% of Arctic plants being polyploids and with frequencies and level of polyploidy strongly increasing northwards within the Arctic (Brochmann *et al.* 2004). This high frequency has also been related to the large-scale climate changes and frequent fragmentation, range expansion and secondary contact between previously isolated entities in the region (Brochmann *et al.* 2004). The evolutionary success of polyploids has been related to their fixed-heterozygous genomes, which buffered against inbreeding and genetic drift through periods of dramatic climate change. In alpine regions, most of the available studies point to a percentage of polyploids higher than 50% (alpine zone of the Mt. Washington, Löve & Löve 1967; Cameroon Mountains, Morton 1993). Still, in the flora of the Hengduan Mountains (Nie *et al.* 2005) and in the alpine flora from Spain (Loureiro *et al.* 2013) much lower values were observed, with only approximately 23% of the analysed taxa being polyploids.

Within angiosperms and similar to the pattern obtained by Otto & Whitton (2000; 31.7% for monocots *versus* 17.7% for dicots), we obtained a higher incidence of polyploids in monocots than in dicots (Fig. 5). This was more evident for the dataset of the Mediterranean region (57.0% *versus* 32.1% for monocots and dicots, respectively), but it was also clear for the other two approaches (55.5% *versus* 46.6%, Fig. 5B, and 59.1% *versus* 44.9%; Fig. 5C).

Our approach also enabled us to explore the occurrence of species with mixed-ploidy, *i.e.* species harbouring two or more ploidy levels as reported in the literature. Despite the low values obtained in the Mediterranean region (most probably because they are based on punctual chromosome counts that miss much of the extant variability), the values obtained for Iberian Peninsula and Andalusia datasets reveal a high variability within species, with almost half of the polyploid species being ploidy-variable in nature (45.0% and 48.7%, for Iberian

Peninsula and Andalusia, respectively; Tables S3 and S4), and thus harbouring cytogenetic diversity that might influence species evolutionary trajectories. This implies that, on average, in the Iberian Peninsula, 23.6% of all ferns and 21.7% of all flowering plants with karyological information harbour multiple ploidy levels, with similar values being observed in the Andalusian subset (22.6% and 23.1%, respectively; Tables S3 and S4). Once again, these values are slightly higher than those obtained by Wood *et al.* (2009) after a broad survey of species (17% for ferns and 12–13% for angiosperms). Monocots also presented higher amounts of species with intraspecific ploidy variation than dicots (33.2% *versus* 19.0%, and 36.3% *versus* 20.1%, for the Iberian Peninsula and Andalusia, respectively; Fig. 5B, C).

Despite our values were overall higher than the estimates given by other authors, we anticipate that even our values are underestimations in comparison with the real occurrence of polyploidy in nature, as most of the species were not studied with sufficient detail, both at fine and large scales. A proof of this is the increasing number of contemporary large-scale cytotype distribution studies that evidenced the occurrence of many taxa with ploidy heterogeneity (Husband *et al.* 2013). Examples from the Mediterranean include *Dianthus broteri* Boiss. & Reut. (Balao *et al.* 2009), *Erysimum mediohispanicum* Polatschek (Muñoz-Pajares *et al.* 2017) and *Limonium* spp. (Caperta *et al.* 2017), among others.

A deeper analysis at the family level revealed that the very well represented families Rosaceae, Caryophyllaceae, Poaceae and Liliaceae *s.l.* are those with a polyploid frequency well above the average. Despite representing a much smaller dataset, the families that stand out in the 1st approach are similar to those observed in the Iberian Peninsula and Andalusian datasets. These results are in accordance with previous works that have already pointed out that polyploidy was an important process in the evolution of these families. In the particular case of Rosaceae, polyploidy has evolved numerous times (Vamosi & Dickinson 2006), with the subfamily Maloideae probably arising following a polyploidisation event (Evans & Campbell 2002). Caryophyllaceae, especially *Silene* L. (Popp *et al.* 2005;

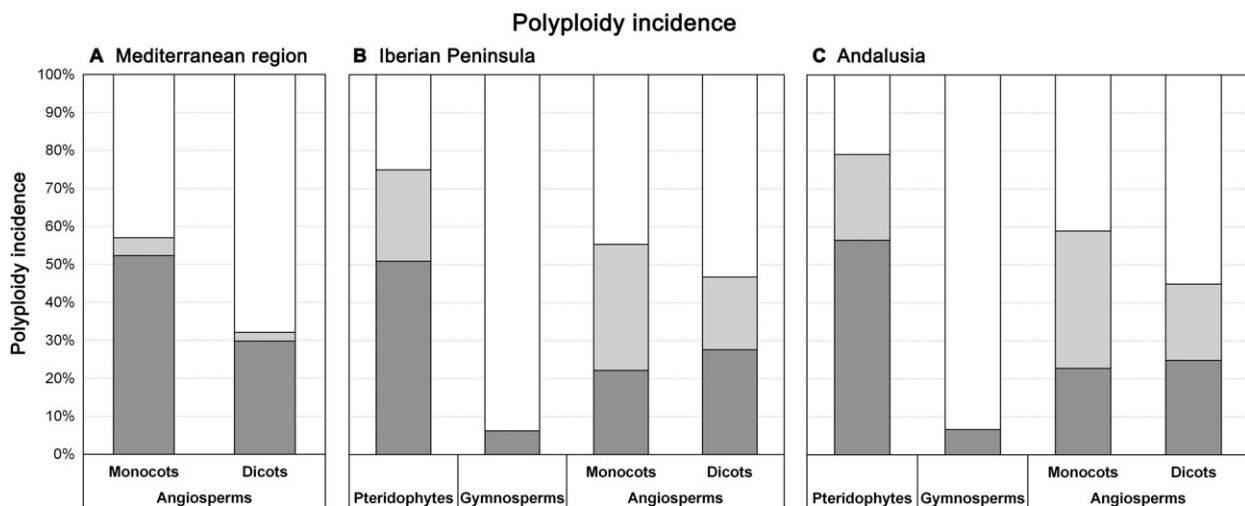


Fig. 5. Frequency of polyploidy in the Mediterranean region (A), Iberian Peninsula (B) and Andalusia region (C). Values are provided as relative percentages, in (A) from the total available information and in (B) and (C) from the total number of species currently recognised. Pteridophytes and Gymnosperms are not represented in the Mediterranean region because of the low amount of available information (3 out of 4 Pteridophytes and 4 out of 5 Gymnosperms were recorded as polyploid).

Popp & Oxelman 2007) and *Arenaria* L. (Favarger & Feliner 1988) genera, has long been regarded as a family comprising polyploid taxa (Blackburn & Morton 1957). As expected from the literature (Levy & Feldman 2002), polyploidy seems to have played a major role in the evolution of Poaceae (46.2% to 69.8% incidence for Iberian Peninsula and Andalusia, respectively; Tables S3 and S4). Genera such as *Festuca* L. and *Anthoxanthum* L. have long been recognised to have a large number of polyploid series (Loureiro *et al.* 2007; Pimentel & Sahuquillo 2007; Šmarda *et al.* 2008; Chumová *et al.* 2015). Our high estimates of polyploidy incidence in Liliaceae *s.l.* (in the first approach combining Asparagaceae, Hyacinthaceae and Liliaceae *s.s.*, altogether with 64.1% of polyploid species; see also Tables S3 and S4 for other approaches) contrasts with previous information available in the literature. Whereas we obtained frequencies ranging between 64.1% and 74.7%, Peruzzi *et al.* (2009) observed significantly lower values (16%) when examining the patterns of chromosome evolution across the family. In the Iberian Peninsula, Liliaceae *s.l.* genera that are well represented in the Mediterranean, such as, *Allium* L., *Asphodelus* L., *Gagea* Salisb. and *Ornithogalum* L., contributed largely to the higher values observed. Boraginaceae (*e.g.* *Myosotis* L.) and Plumbaginaceae (*e.g.* *Limonium*) also presented frequency values above the average. Despite no exhaustive study being made to evaluate the frequency of polyploidy in such families or genera, there are several pieces of evidence in the literature that support our estimations (Štěpánková 2001; Castro & Rosselló 2007; Caperta *et al.* 2017). The family of Asteraceae is also noteworthy given its representation in the region. Polyploidy has long been recognised as a frequent mechanism in Asteraceae (Mota *et al.* 2016 and references therein) and the frequency of polyploid species observed for the family, although low for the 1st approach, was fairly high for the Iberian Peninsula and Andalusia (46.0% and 44.1%, respectively), with genera such as *Hieracium* L., *Leucanthemum* Mill., *Senecio* L., *Taraxacum* Zinn, *Centaurea* L. and *Artemisia* L. having numerous polyploid species.

Although being represented in the Iberian Peninsula and Andalusia by a much smaller number of taxa, there are families where polyploidy also appears to be an important process. These include, for example, Crassulaceae, Malvaceae, Papaveraceae and Orchidaceae. In the last family, polyploidy has been suggested to contribute to the evolutionary diversification in the Iberian Peninsula (Amich *et al.* 2007), and previous estimates for this region (39.7% infrageneric polyploids; Amich *et al.* 2007) are similar to those obtained here (42.7%; Table S3). Still, this family is very complex cytogenetically, as besides the occurrence of polyploidy, other phenomena such as hybridisation, aneuploidy and dysploidy have been frequently reported (Bianco *et al.* 1991; Amich *et al.* 2007), and, thus, it deserves further studies in the future. There are also important families of the Mediterranean ecosystems where polyploidy seems to be less frequent. These include the Apiaceae (Umbelliferae), Cyperaceae, Ericaceae, Euphorbiaceae and Thymelaeaceae.

Among the families that impressed by the large percentage of mixed ploidy taxa, Crassulaceae, Liliaceae, Rubiaceae and Poaceae clearly appear as much above the average. Other families with high ploidy incidence, where multiple ploidy levels are also described for more than half of the polyploid species, include the Salicaceae, Polygonaceae, Asteraceae and

Amaryllidaceae, among others. In Linaceae, Dipsacaceae and Ericaceae, despite polyploidy incidence being low, it is interesting to note that the few polyploid species are ploidy-variable. Members of all these families clearly merit further large-scale screenings as they may reveal important study systems to evaluate the emergence and maintenance of polyploid taxa in this region.

When we compare the incidence of both hybridisation and polyploidy at the family level, we observe that in most cases, families where hybridisation seems to prevail, polyploidy was also an important phenomenon (*e.g.* Amaryllidaceae, Orchidaceae, Plumbaginaceae, Salicaceae, Violaceae). One of the only exceptions is the family Fagaceae, where the frequency of hybridisation was very high, but no polyploids have been reported so far. Some genera where both phenomena seem important are *Narcissus* and *Limonium*. In the particular case of *Narcissus*, species from this genus present a remarkable karyological variability with two basic chromosome numbers ($x = 5$ and $x = 7$) and several derived ones (*e.g.* $x = 11$), as a result of chromosomal rearrangements, hybridisation and polyploidy, even between species not closely related (Fernandes 1968; Marques *et al.* 2011, 2017). Several triploid natural hybrids are also known. These seem to benefit from strategies such as bulb propagation to establish themselves in populations (Marques, unpublished results). *Limonium* is also a taxonomically complex group, punctuated with hybridisation and polyploidy, with these phenomena being associated with uniparental reproduction, including self-fertilisation, agamospermy and gynogenesis (Caperta *et al.* 2017).

Isolation barriers in Mediterranean plant groups

Numerous studies have documented sympatric populations where different entities coexist. As described above, the coexistence of homoploid hybrids or neopolyploids with their parental taxa can either be a transition step where the new entities are recurrently formed or a persistent stage if a series of isolation barriers mediate assortative reproduction (Felber 1991; Petit *et al.* 1999). Such reproductive barriers include different microhabitat preferences (Felber-Girard *et al.* 1996; Baack 2005), mechanical isolation due to differences in floral morphology (Grant 1994), flowering time divergence (Petit *et al.* 1997; Jersáková *et al.* 2010), assortative mating mediated by different pollinator behaviour or by different pollinator species (Grant 1994; Thompson & Merg 2008) and/or intrinsic characteristics of the breeding system (*e.g.* breakdown of self-incompatibility under mixed pollen loads; Mráz 2003), among others. These isolation barriers will govern the levels of pollen flow and determine the fate of the new lineages (Hewitt 1988; Lexer & van Loo 2006).

However, how much do we know about the role of isolation barriers in shaping the patterns of hybridisation and polyploidy in Mediterranean plants? The hybrid and polyploid entities that occur in the Mediterranean region are very poorly studied from an ecological point of view. First, only a few studies have focused on the distribution patterns at various scales of the Mediterranean polyploid species (*e.g.* Lumaret *et al.* 1987; Mansion *et al.* 2005; Buggs & Pannell 2006, 2007; Balao *et al.* 2009; Manzaneda *et al.* 2012; Caperta *et al.* 2017), making it difficult to depict and interpret geographic patterns and delineate contact zones to study interaction dynamics between

parentals and the new entities. Second, in this region, even fewer studies have evaluated the role of isolation barriers between hybrids and their progenitors or in polyploid complexes. In Table S5, we present a review of the few published studies, in an attempt to decipher the type (pre- and/or post-pollination), role and importance of barriers in promoting the isolation of new lineages in the Mediterranean. Despite finding several studies that suggested an important role of some specific barrier, only a few works tried to actually quantify the strength of such isolation barriers between species/cytotypes (Table S5). Additionally, most studies focused on specific genera such as *Orchis* (and other orchids; Pellegrino *et al.* 2005, 2010; Xu *et al.* 2011; Luca *et al.* 2015) and *Narcissus* (Marques *et al.* 2007, 2012), which reveals that our knowledge about the role of isolation barriers is poorly understood for most of the hybridising Mediterranean groups. It was even harder to find studies devoted to the study of isolation barriers in polyploids. An example is the allopolyploid wind-pollinated *Mercurialis annua* L. that presented highly dynamic moving zones. In this polyploid complex, no reproductive barriers prevent reproduction and higher reproductive success of diploids lead to asymmetrical pollen swamping and higher hybrid production in the hexaploids, which are gradually displaced by diploids each year (Buggs & Pannell 2006). Although most studies did not explicitly quantify the reproductive isolation between different cytotypes, it is clear that geographic barriers seem to be important in most polyploid systems studied so far (Table S5), with polyploids and lower ploidy progenitors occupying different geographic ranges. Still, numerous contact zones where two or more entities coexist are being increasingly detected in nature, and the role of the other isolation barriers is still largely unexplored.

The importance of ecological segregation in speciation is also a main factor that limits or prevents gene flow among new sympatric lineages. As stated above, hybridisation and polyploidy could mediate niche shifts resulting from new tolerances, with the new entities being able to colonise new habitats and spread beyond their progenitor populations. Indeed, several studies have reported, for example, differences between diploid and established polyploids in the ecophysiological tolerance to abiotic stresses (*e.g.* Levin 2002; Maherali *et al.* 2009; Hao *et al.* 2013). Consequently, the range of parental species and polyploids is expected to differ, and polyploids have been often postulated to have larger geographic ranges (Levin 2002; Lowry & Lester 2006; but see Martin & Husband 2009) and to grow in more extreme environmental conditions (*e.g.* Brochmann *et al.* 2004). Several Euro-Mediterranean plant groups also revealed a wide distribution range of polyploids, with diploids presenting fragmented and smaller ranges than polyploids (reviewed in Thompson 2005). Some studies have also suggested a high frequency of polyploids at high latitudes, high altitudes and in recently glaciated areas (reviewed in Levin 2002; Brochmann *et al.* 2004; Vamوسي & McEwen 2013) although this idea still needs to be tested in the Mediterranean region. For example, the distribution of allo- and autopolyploids, and homoploid hybrid species along an elevation gradient revealed that polyploids (especially those of hybrid origin) are disproportionately present at high elevations (Vamوسي & McEwen 2013). A higher frequency of hybrids, often stabilised *via* polyploidisation, has also been observed in the Arctic flora, being shaped by large-scale climate changes

that resulted in cycles of fragmentation, range expansion and reunion of previously isolated populations (Brochmann *et al.* 2004). In contrast, no significant variation in the ecological amplitude between diploids and tetraploids was found in flora from the Pyrenees (Petit & Thompson 1999), or in diploid and tetraploid endemics from Corsica occurring in similar altitudinal belts (Contandriopoulos 1962). Ecological differentiation has been studied in specific polyploid complexes, with a few studies in Mediterranean groups. For example, in *Arrhenatherum elatius* (L.) J.Presl & C.Presl, differentiation among tetraploid populations enabled the spread of the polyploid cytotype into different environments (Petit & Thompson 1997). In *Mercurialis annua*, reciprocal transplants and experiments under controlled conditions revealed an ecophysiological superiority of the diploids, suggesting that they are preadapted to the (contact) area currently being colonised (Buggs & Pannell 2007). Also, in *Dactylis glomerata* L. cytotypes presented distinct physiological requirements, related to habitat differentiation (Lumaret *et al.* 1987). Finally, differences in water-use efficiency were detected within *Brachypodium distachyon* (L.) P. Beauv., with the distribution of diploids and tetraploids being structured by aridity gradients (Manzaneda *et al.* 2012, 2015).

FUTURE PERSPECTIVES

Phylogenies and analyses of plant genomes exposed the importance of interspecific hybridisation and polyploidy in the evolutionary history of plants and, in the last decades, significant insights have been gathered on the genetic, epigenetic and genomic consequences of hybridisation and genome duplications (Soltis *et al.* 2010, 2016). Nevertheless, many questions remain to be addressed, even in a well-studied botanical area such as the Mediterranean region. Remarkably, several Mediterranean plant studies have used hybridisation as an ‘*abracadabra/hocus pocus*’ explanation for the lack of patterns or for the phylogenetic incongruence found between different inherited markers. But, as shown here, our knowledge about the frequency of hybridisation and polyploidy in this region is still quite limited and reflects the need for further studies. Despite having potential shortcomings, our quantifications on the frequency of hybrids and polyploids in the Mediterranean region are similar to the frequencies reported for other geographic areas and even revealed to be relatively high in the Iberian Peninsula. As expected, hybrids and polyploids were non-randomly distributed taxonomically, being more prevalent in certain families and genera. Such groups might be viewed as important in terms of recent hybridisation, although we should also acknowledge the presence of taxonomic biases, as some plant groups have traditionally received more attention than others. Still, we are aware that our approaches have limitations and that the quantifications reported here should be regarded as baseline numbers. Our estimations might change in the coming years with the development of more accurate Flora monographs across the Mediterranean Basin that should include a full description of the hybrids, as well as making the data openly available and with georeferenced localities. Regardless of their pervasiveness in nature, the knowledge on cytotype distribution is also extremely limited to a few polyploid complexes that have been studied in more detail, or limited to a few estimates (usually poorly georeferenced) per taxon, which

largely restricts our knowledge on the general distribution patterns of polyploids. Thus, considering the huge barrier that ploidy changes might impose to gene flow, future taxonomic studies should also include this additional level of cryptic diversity.

Future genomic and chromosomal evolution studies will also affect our estimations, as they continue to reveal that numerous plants (e.g. Jiao *et al.* 2011; Amborella Genome Project 2013; Li *et al.* 2015; Barker *et al.* 2016b; Mota *et al.* 2016), animals (e.g. Clarke *et al.* 2015; Pasquier *et al.* 2016) and other eukaryotic genomes (e.g. Conant 2014; Scannell *et al.* 2006) have experienced ancient polyploidy events during their evolutionary history, with some polyploid organisms currently behaving as diploids (e.g. Barker *et al.* 2012; Edger *et al.* 2015; Barker *et al.* 2016b and references there in; Marques *et al.* 2016). Large-scale cytogenetic studies will also increase our understanding of contemporary polyploid groups and genome duplication events (Ramsey & Ramsey 2014), providing novel insights on the nature of contact zones and on the ecological factors driving speciation (e.g. Petit *et al.* 1999; Stuessy *et al.* 2004; Kolář *et al.* 2009).

Currently, and despite the advances found through genomic studies, we were surprised to see that critical areas of research such as the ecology, reproduction and physiology of hybrids and polyploids have received significantly less attention in the Mediterranean region (but see the examples provided in Table S5). These areas are crucial to understand the ecological determinants involved in hybrid and polyploid formation, establishment and spread in nature (Thompson & Lumaret 1992). Many studies have also revealed the importance of isolation barriers to prevent gene flow between species (Coyne & Orr 2004); nevertheless our knowledge on the impact of hybridisation and polyploidy in the occurrence of these barriers, on how these processes affect the ecological interactions and on how they promote the origin of novel traits is still limited. Although recent studies, using synthesised or naturally occurring neopolyploids, start to be able to decouple the effects of genome duplications from the evolutionary changes that have occurred after the emergence of the new entity (e.g. Ramsey & Schemske 2002; Husband *et al.* 2008; Ramsey 2011), the available information is still scarce. Thus, the question remains: what is role of hybridisation or genome duplications *per se* in the successful establishment of new entities?

Many traditional dogmas are also being challenged. For instance, although polyploidy has been proposed as a mechanism of ‘instant speciation’ (Lutz 1907; Winge 1917), some recent studies revealed that the reality is less straightforward (Husband & Sabara 2004; Husband *et al.* 2016; Vallejo-Marín *et al.* 2016), and there are now many challenging questions on how genome duplication might contribute to reproductive isolation and speciation (Soltis *et al.* 2010, 2016). The general idea that hybrids always threaten the persistence of parental species (Levin *et al.* 1996) also needs to be re-evaluated. When hybrids

arise, they face major difficulties since the newly formed diploids and triploids are basically sterile and, even when an even-numbered chromosome number is achieved, chromosome pairing is hardly ever perfect (Ramsey & Schemske 2002). Thus, how many hybrids arise but rapidly go extinct due to competition? And how many are threatened and, in that case, should they be conserved (Thompson *et al.* 2018)? Overall, these questions remind us of the many gaps that still need to be addressed in this dynamic field. With the rise of genomic methods and deep-field ecological and morphological studies, we expect to see many of these questions addressed in the future, enabling us to better understand the role of hybridisation and polyploidy in the Mediterranean, and worldwide.

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SUPPORTING INFORMATION

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

Table S1. List of families where hybrid taxa have been recorded according to *Flora iberica* (Castroviejo 1986–2014).

Table S2. List of genera growing in the Iberian Peninsula where hybrid taxa have been recorded according to *Flora iberica* (Castroviejo 1986–2014).

Table S3. Frequency of polyploidy in the most representative Iberian Peninsula angiosperms families (i.e. families with 20 or more species).

Table S4. Frequency of polyploidy in the most representative Andalusian angiosperms families (i.e. families with 20 or more species).

Table S5. Examples of isolation barriers in Mediterranean plants.

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