Spatial conservation prioritisation for intraspecific genetic diversity of three forest tree species with a fragmented distribution in Italy

Elia Vajana[a,*], Marco Andrello[b], Camilla Avanzi[a], Francesca Bagnoli[a], Giovanni G. Vendramin[a], Andrea Piotti[a]

[a] Institute of Biosciences and BioResources (IBBR), National Research Council (CNR), 50019 Sesto Fiorentino (Firenze), Italy

[b] Institute for the study of Anthropic impacts and Sustainability in the marine environment, National Research Council, CNR-IAS, Rome, Italy

* Corresponding author. *E-mail address*: elia.vajana@ibbr.cnr.it. *Full postal address*: Institute of Biosciences and Bioresources, National Research Council of Italy, Via Madonna del Piano 10, 50019 Sesto Fiorentino (Firenze), Italy.

Abstract

The Kunming-Montreal Global Biodiversity Framework recognised the urgency of taking action to conserve intraspecific genetic diversity (IGD) as an insurance against habitat degradation and environmental change. Recent work suggests that 90-99% of IGD should be conserved to safeguard viability of future generations.

Here, we addressed such conservation issue in three forest tree species in Italy: silver fir (*Abies alba* Mill.), Heldreich's pine (*Pinus heldreichii* H. Christ), and pedunculate oak (*Quercus robur* L.). We used microsatellite markers to measure IGD of 36 (*A. alba*), 15 (*P. heldreichii*) and 25 (*Q. robur*) natural sites, including several putative glacial refugia. We developed a Spatial Conservation Prioritisation (SCP) analysis to quantify the genetic irreplaceability of each site and identify the minimum set coverage ensuring IGD protection. Finally, we compared SCP results with the contributions to diversity within and between sites, total allelic richness and private allelic richness.

We found that between 44% and 73% of sites were required to conserve 90-99% of the alleles, and that this conservation effort held even when targeting lower percentages of alleles to protect (50-75%). Glacial refugia were often included in the minimum set coverage, confirming theoretical and biogeographical expectations. Finally, sites with high genetic irreplaceability were found to have higher private allelic richness on average. These results are discussed in the light of the biogeographic history of the species studied and the current policies for the conservation of forest genetic resources.

Keywords: Spatial conservation prioritisation; forest genetic resources; intraspecific genetic diversity; *Abies alba*; *Pinus heldreichii*; *Quercus robur*.

1. Introduction

Intraspecific genetic diversity (IGD), encompassing variation within and between populations, is a key component of ecosystem functioning as it underlies the species' capacity to adapt to the environment, survive environmental change and provide human society with ecosystem services (Robuchon et al., 2022). Particularly, IGD of forest trees (*aka* forest genetic resources) contributes to climate regulation, water filtration, nutrient cycling, wood production, and coastal and soil protection against erosion (Alberto et al., 2013; Gougherty et al., 2021; Panagos et al., 2015; Schweitzer et al., 2011). The preservation of forest genetic resources lies at the core of sustainable forest management and conservation (Fady et al., 2016). Yet, societal activities inducing global warming, habitat fragmentation and overharvesting are accelerating biodiversity decline worldwide, with massive losses in genetic diversity and unpredictable impacts on the biosphere (Exposito-Alonso et al., 2022; Hoban et al., 2023). As a consequence, between 16-66% of the whole amount of IGD on Earth is estimated to go lost in the next decades unless specific actions are undertaken to halt genetic erosion and sustain evolutionary rescue (Hoban et al., 2023, 2021).

Among the long-term goals for 2050, the Kunming-Montreal global biodiversity framework formally recognized the need of conserving IGD and the urgency of putting in place actions to protect 'the genetic diversity within and between populations of native, wild, and domesticated species to maintain their adaptive potential' (Convention on Biological Diversity, 2022; Hoban et al., 2023). To this aim, the conservation of 90-99% extant IGD is usually advocated together with guaranteeing an adequate effective population size (*Ne*) so as to avoid promoting genetic erosion and prevent 'future losses for all populations [...] regardless of past losses' (Frankham, 2022; Hoban et al., 2023).

Establishing Conservation areas is among the strategies proposed to contrast genetic erosion (Hoban et al., 2023; Robuchon et al., 2021). However, budget constraints usually require choices to be made when attempting to preserve IGD with such a strategy (Vane-Wright et al., 1991; Weitzman, 1998; Marris, 2007). Then, scientifically based criteria were developed for setting spatial priorities for conservation, e.g., whether to prioritise genetically distinct vs. highly diverse populations (Fernandez-Fournier et al., 2021; Petit et al., 1998; Weitzman, 1993) or sites with endemic lineages rather than centres of radiation (Erwin, 1991; Robuchon et al., 2021). More recently, spatial conservation prioritisation (SCP) was applied to optimise spatial planning for new conservation areas based on genetic information (Andrello et al., 2022; Nielsen et al., 2023b). In this case, spatially optimised solutions are found across the study area to minimise the costs required to achieve specific conservation objectives for IGD, a formulation of the SCP problem known as 'minimum set coverage' (Moilanen et al., 2009).

Here, we used SCP to protect IGD in three keystone forest tree species characterised by a highly fragmented distribution in Italy – two mountainous conifers, i.e., silver fir (*Abies alba* Mil.) and Heldreich's pine (*Pinus heldreichii* H. Christ), and a broadleaved lowland tree, the pedunculate oak (*Quercus robur* L.). Palaeobotanical and genetic evidence proved that the Mediterranean region provided temperate trees with climatically favourable refugial areas during the glacial periods characterising the Quaternary (Petit et al., 2003). Such glacial refugia were mainly found in the Iberian, Italian and Balkan peninsulas, with populations inhabiting these areas generally harbouring high levels of IGD and/or genetic distinctiveness

(Hewitt, 1996; Petit et al., 2003). Such genetic features make forest tree populations from former glacial refugia candidate sites for conserving forest genetic resources, as they could be rich in unique variation that might prove necessary to foster adaptive potential in the face of climate change (Petit et al., 2005). Here, we focused our analysis on a set of sites within a complex of refugial areas (Brewer et al., 2002; Petit et al., 2002; Piotti et al., 2017), where fragmentation is likely pushing peripheral populations towards an extinction vortex in concert with climate change (Bucci et al., 1997; Gibbs, 1997; Maiorano et al., 2013; Ruosch et al., 2016; Thomas et al., 2002).

The goals of the present work were (i) to identify priority sites for conserving IGD in silver fir, Heldreich's pine and pedunculate oak in a multi-refugial area, (ii) to explore the effect of varying conservation effort (i.e., number of sites protected) on the amount of IGD secured, as well as (iii) to investigate the consensus between priority sites identified through SCP and those found through other genetic metrics based on allelic richness, an indicator of genetic health sensitive to recent demographic changes.

2. Materials and methods

2.1. Sampling and genotyping

We relied on genetic data from 36 natural populations of *A. alba* (seven of which were specifically characterised for this study, resulting in 1769 trees in total, 49 on average per site; **Supplementary Table 1**), 15 natural populations of *P. heldreichii* (515 trees, 34 on average), and 25 natural populations of *Q. robur* (745 trees, 30 on average) to feed SCP analyses (**Figure 1**). The large representativeness of IGD was guaranteed by densely covering the local distribution of the species, and by including sampling sites from all known putative glacial refugial areas.

A. alba was genotyped at 16 nuclear short sequence repeats (nSSRs), resulting in 145 alleles (Piotti et al., 2017; Santini et al., 2018), *P. heldreichii* at 12 nSSRs (89 alleles; Piovesan et al., 2023), and *Q. robur* at 16 nSSRs (245 alleles; Avanzi et al., 2023). The details about genotyping and standard population genetic analyses can be found in the above mentioned references.

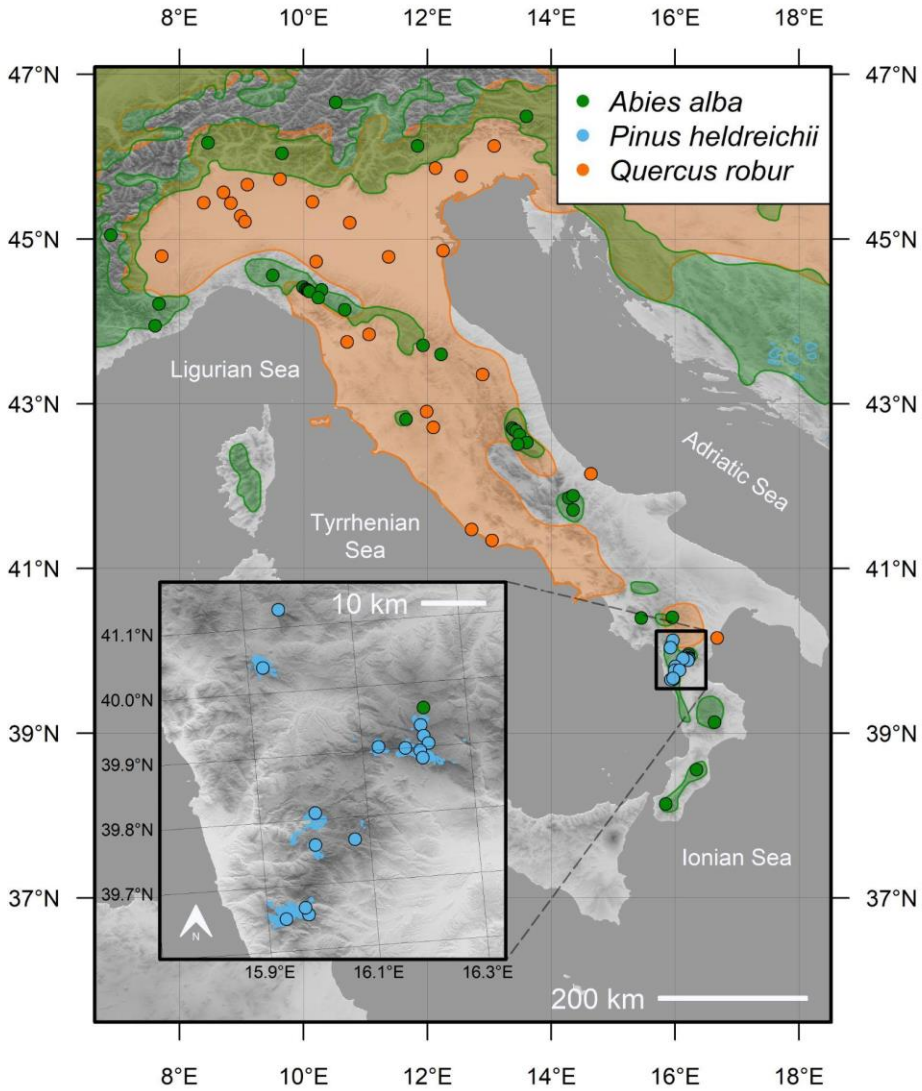

Figure 1. Sampling sites: *Abies alba* (green points), *Pinus heldreichii* (blue points), and *Quercus robur* (orange points). Approximate species distributions are also shown in the background with the same colour scheme (Caudullo et al., 2017). The inset map zooms over the Pollino National Park, which encircles the distribution of Heldreich's pine in Italy.

2.2. Spatial conservation prioritisation

The SCP analysis was run separately for each species, as there were no common sampling sites. In each species, each allele scored was considered as a distinct conservation feature (see the 'AL' method in Andrello et al., 2022). The spatial distribution of each allele was coded as presence/absence in each site. The number of selected sites was considered as a simple proxy for total conservation cost (Paz-Vinas et al., 2018; von Takach et al., 2021). The objective was to find solutions for conserving low (50%), moderate (75%) and high (90%, 99% and 100%) proportions of protected alleles. These proportions of protected allele were interpreted as levels of protected IGD (Paz-Vinas et al., 2018), and included the 90% objective proposed in the first draft of the post-2020 GBF (CBD, 2021), as well as the 99% objective proposed more recently (Frankham, 2022; Hoban et al., 2023).

The SCP was run under the 'minimum set coverage' formulation to minimise the total conservation cost needed to achieve a given level of IGD protection. This was operationalized as finding the minimum set of sites ensuring that the given proportion of alleles (i.e., 50%, 75%, 90%, 99% or 100%) was adequately protected. An allele was considered as adequately protected if at least 30% of its occurrence sites were included in the solution as an insurance against catastrophic events (Lefèvre et al., 2020; Wilson et al., 2009). For the 50%, 75%, 90% and 99% IGD levels, we performed 100 independent replicates, each based on a random selection of alleles to be protected. We calculated the genetic irreplaceability (GI) of each site as the number of times each site was selected across the 100 replicates (Pressey et al., 1994). For the 100% IGD level, a single analysis was performed as all the alleles were included by definition, which allowed the prioritisation problem to find a unique, optimal set of sites needed to conserve all the alleles. All SCP analyses were performed using the R package 'prioritizer' v. 7.2.2 (Hanson et al., 2022) and solved within 10% optimality using the 'Gurobi' solver v. 9.5.2 (Gurobi Optimization, LLC, 2023).

2.3. Relationship between genetic irreplaceability and genetic diversity metrics

We studied the relationship between GI and four genetic diversity metrics commonly used to prioritise populations for conservation: the site-specific contribution to within (A_S) and between sites (D_A) diversity (i.e., the mean number of alleles occurring in the sites under study, and the mean number of alleles occurring in a site and absent in another, respectively), the contribution to total allelic diversity (A_T; the sum of A_S and D_A, i.e., a measure of the overall allelic diversity across sites), as well as private allelic richness (*PAr*; the number of unique alleles in a given site). Following this approach, sites mostly contributing to A_T can be identified by their relative contribution to A_S and D_A (Petit et al., 1998). A_T, A_S and D_A were calculated for all sites in each species with the 'metapop2' software (López- Cortegano et al., 2019). *PAr* was estimated with 'HP-RARE' (Kalinowski, 2005). Rarefaction was used to account for uneven sample sizes in allelic richness calculation (El Mousadik and Petit, 1996).

Considering the strongly bimodal distributions of GI values obtained when conserving 99% of alleles, populations were classified to be at low/high GI (see section 2.2.1), using a threshold of ≤20% (low GI) and ≥80% (high GI), respectively. Associations between levels of GI and genetic metrics were tested through either the Welch or Student's two sample *t*-test, depending on whether the variances of the groups resulted to be unequal or equal based on the Levene's test. To reduce inflation in the familywise type I error due to multiple testing, the Bonferroni correction was applied to the *p*-values of the tests involving the same species. A test was deemed to be statistically significant if its adjusted *p*-value was equal to or lower than the nominal significance threshold (α) of 0.05. All operations were performed in the R programming environment (R Core Team, 2022).

3. Results

3.1. Spatial conservation prioritisation

In all species, the number of sites needed for conserving 100% IGD was less than the total number of sampled sites, and was equal to 16 (44% of sampled sites) in *A. alba*, 11 (73%) in

P. heldreichii, and 18 (72%) in *Q. robur*. For conserving lower levels of IGD, the median number of sites needed was equal or slightly lower with almost no variation among the replicates (**Table 1**).

Table 1. Median number of sites needed to conserve 50%, 75%, 90%, 99%, and 100% of IGD across 100 replicates in *Abies alba*, *Pinus heldreichii* and *Quercus robur*.

	Protection levels				
	50%	**75%**	**90%**	**99%**	**100%**
A. alba	14 (±1)[a]	15 (ND)[b]	16 (ND)	16 (ND)	16[c]
P. heldreichii	8 (±1)	10 (±1)	11 (ND)	11 (ND)	11
Q. robur	15 (±1)	17 (±1)	18 (ND)	18 (ND)	18

[a] Median absolute deviation (MAD), i.e., an estimate of the variability around the number of sites in the replicates. Each MAD was calculated as the median of the absolute deviations from the number of sites. [b] No deviation from the number of sites observed. [c] No variation was associated with the 100% level as a unique analysis was performed in this scenario.

Regardless of the number of alleles to be protected in the SCP analysis, we found that ~98% of the replicates conserved at least 90% of all alleles present in the dataset (**Figure 2**). All replicates protected >90% of alleles present in the dataset when including 75% of alleles in the SCP analysis. Overall, the SCP analyses systematically ensured protection for higher numbers of alleles than those included as conservation features.

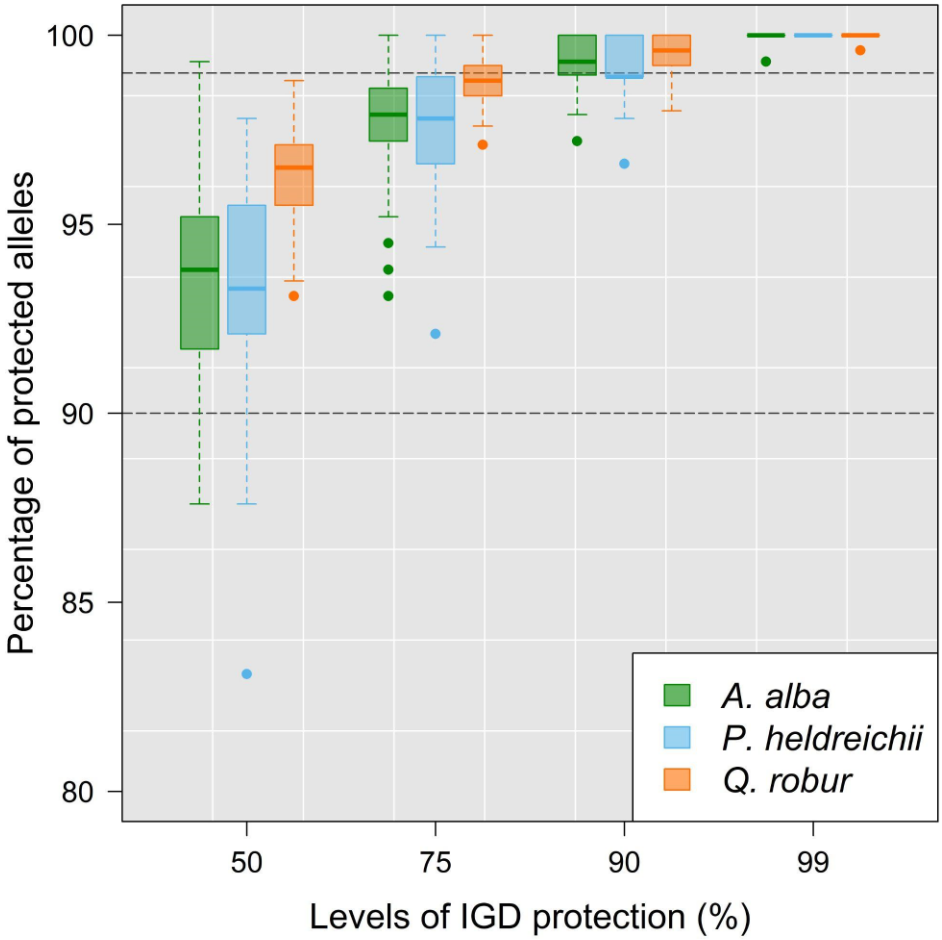

Figure 2. Variation of the percentage of alleles conserved in at least 30% of their sites of occurrence as a function of levels of IGD secured (50%, 75%, 90%,99% and 100%). The proportion of alleles adequately protected is represented separately for each species.

For *A. alba*, a bimodal spatial pattern of GI appeared and four sites - two in the northern Apennines and two in the southern Apennines - were irreplaceable (i.e., GI=100%) when 90% of the alleles were conserved (**Figure 3a**). When targeting the 99% level, 11 sites became irreplaceable and were more evenly distributed across the Italian peninsula (**Figure 3b**). In this case, about half of such sites (five out eleven) were located in the southern Apennines. For *P. heldreichii*, 11 sites showed GI≥88% when conserving ≥90% of alleles, including one irreplaceable site on the southern margins (**Figure 3c**). When 99% of the alleles were conserved, all such 11 sites became irreplaceable (**Figure 3d**). Irreplaceable sites were located at short distances (<10 Km apart), and included a group of five close, geographically central sites, and three sites on the southern margins. For *Q. robur*, we found three irreplaceable sites even when conserving 75% of alleles (result not shown), including 'Bosco Pantano', the southernmost site in Italy hosting a unique genetic lineage. When protecting 90% of alleles (**Figure 3e**), four sites were found to be irreplaceable, including one new site in the Po valley (northern Italy). In this case, we found 18 sites with GI≥68%, and seven sites with GI≤27%. The same sites at higher GI were confirmed when conserving 99% of the alleles, when we observed a GI higher than 96% and lower than 5% in sites at high vs. low GI, respectively (**Figure 3f**).

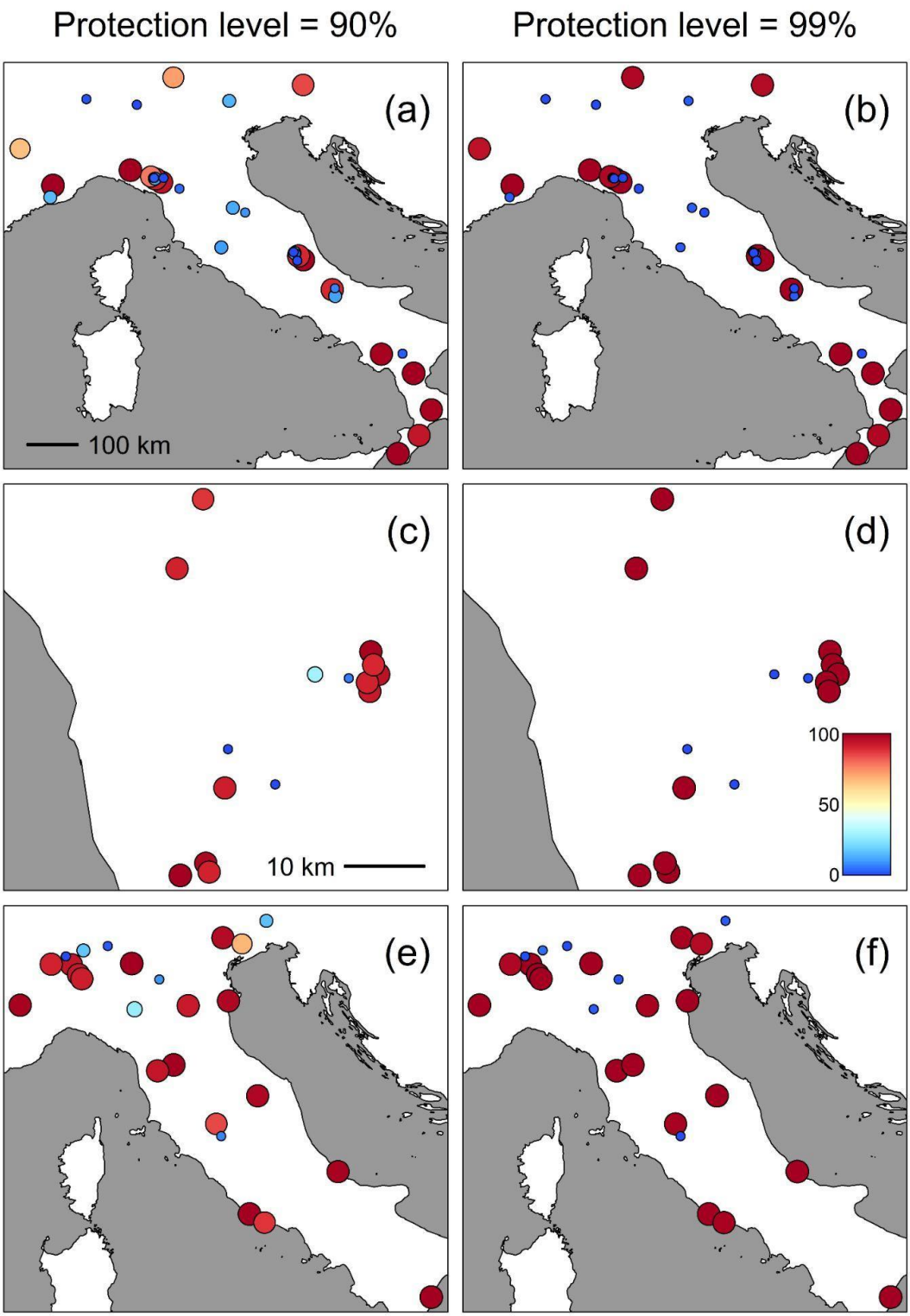

Figure 3. Genetic irreplaceability (GI) of sampled sites in *A. alba* (**a,b**), *P. heldreichii* (**c,d**), and *Q. robur* (**e,f**), as calculated over 100 replicates for different levels of IGD secured (i.e., 90% and 99% on the left and right columns, respectively). Blue tones indicate low GI, while red tones indicate high GI.

3.2. Relationship between genetic irreplaceability and diversity metrics

The relationship between GI and allelic richness components was assessed based on the GI values obtained when protecting 99% of alleles. After multiple testing corrections, four out of

12 tests resulted to be statistically significant at a nominal α=0.05 (**Figure 4**). Particularly, *PAr* resulted to be associated with GI in all species, with the set of sites at higher GI showing a higher mean *PAr*. *PAr* showed high variability in sites with high GI (**Figure 4** and **Supplementary Table 2**). Neither A_T nor A_S resulted to be associated with GI. On the contrary, D_A turned out to be significantly associated with GI in *A. alba*, where highly irreplaceable sites showed to contribute to a larger extent to differentiation in allelic diversity.

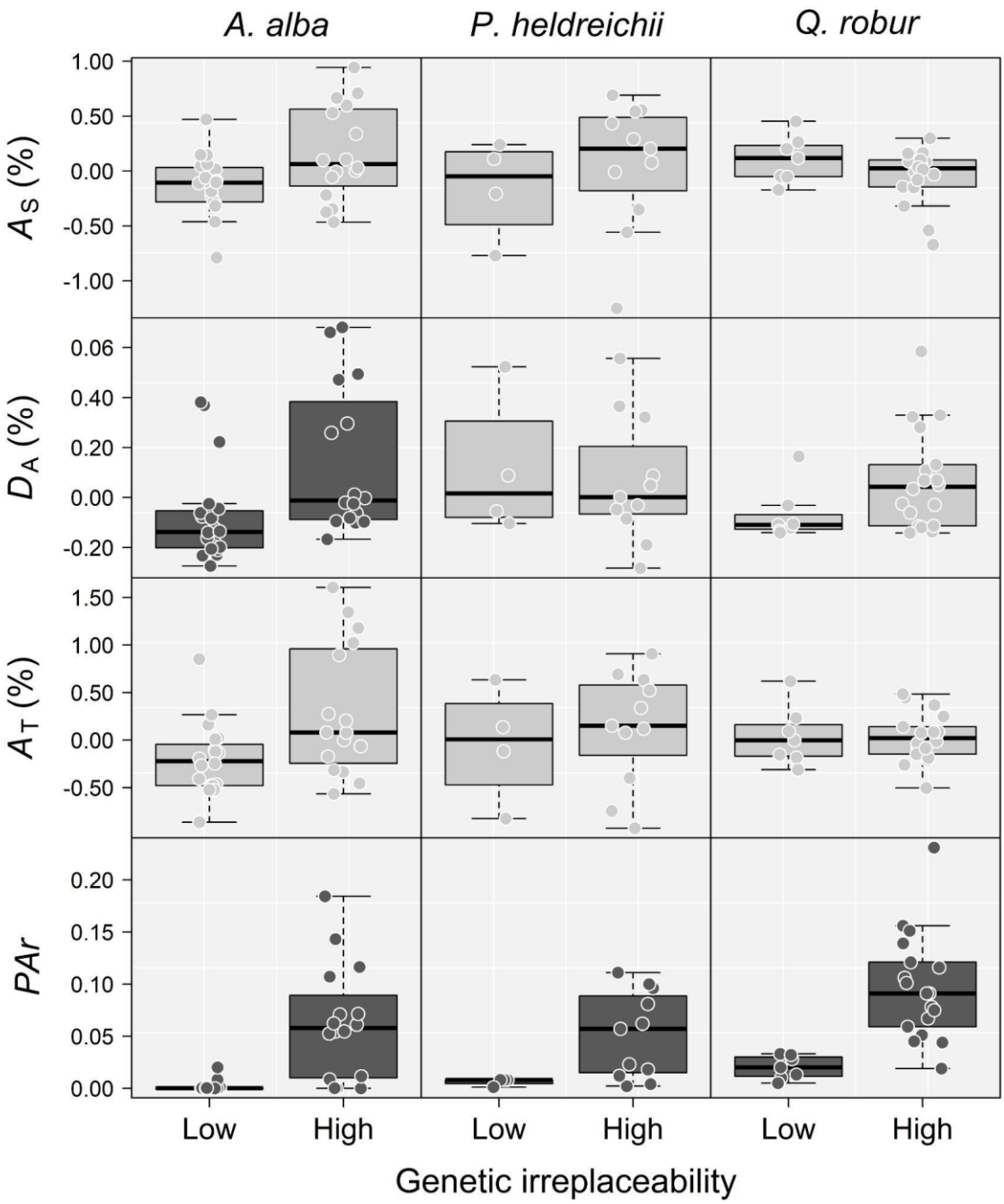

Figure 4. Boxplots of the site-specific contributions to within sites diversity (A_S), between sites diversity (D_A), total allelic diversity (A_T), and private allelic richness (*PAr*) within the groups of sites at low and high genetic irreplaceability (GI<20% and GI>80%, respectively). Darker panels point to statistically significant differences between the means of the sites at low and high GI after the Bonferroni correction for multiple testing.

4. Discussion

We applied SCP to genetic data covering the Italian distribution of three forest species that are key elements of Mediterranean forest ecosystems (*Abies alba*, *Quercus robur*, and *Pinus heldreichii*). Overall, we found that between 44% and 73%of the sampled sites, depending on the species, was needed to achieve adequate protection for all the alleles present in the sites studied. Our analyses showed that even if the objective was protecting fewer alleles (50% of alleles), the required number of sites did not change much. Moreover, even when the objective was to protect fewer alleles, the selected sites could ensure protection for a higher proportion of alleles. Finally, we found that private allelic richness (i.e., alleles present in a single site) was the genetic index mostly related to genetic irreplaceability (i.e., the selection frequency of sites to be protected across replicates). In the following, we discuss these results in the light of the conservation status of silver fir, Heldreich's pine and pedunculate oak, as well as their life history traits and biogeographic history.

4.1. Full protection can be achieved at 'little' extra cost

Our SCP analysis resulted in finding a rather costly solution to protect IGD to the suggested level of 99% (Frankham, 2022). In fact, the percentage of protected sites was never less than 44% and included 16, 11 and 18 sites for silver fir, Heldreich's pine and pedunculate oak, respectively (**Table 1**). Nevertheless, a difference of only two to three sites separated the achievement of half and full protection of alleles, suggesting that low levels of protection (50% of alleles) are neither a parsimonious strategy for IGD conservation, nor do they imply substantial cost optimization. Yet, any additional costs of achieving the 99% protection level (e.g., land acquisition, management and associated opportunity costs) will depend critically on the available budget (Naidoo et al., 2006).

4.2. Private allelic richness is not a perfect proxy for genetic irreplaceability

To explore consensus between different approaches to priority setting, we compared the genetic irreplaceability of a site with the relative contribution of the site to within- and between sites diversity (A_S and D_A, respectively), total allelic diversity (A_T), as well as private allelic richness (*PAr*). Such metrics find wide application in conservation thanks to their sensitivity to fragmentation, genetic bottlenecks and population size (López-Cortegano et al., 2019; Petit et al., 1998; Tapio et al., 2006), and their capacity to comply with the principle of complementarity (Bonin et al., 2007) and to inform allocation of economic resources (Von Takach et al., 2023). The significant, positive correlation found between GI and *PAr* points to the spatial distribution of unique alleles as one of the main genetic features driving site selection in SCP (**Supplementary Figure 1**). By relying on an approach similar to that used here, selection for sites enriched in private alleles has also been observed in other taxa, including freshwater fish species (Paz-Vinas et al., 2018) and marsupials (Von Takach et al., 2023). However, such a correspondence between GI and *PAr* is not straightforward, as we found that some sites with low *PAr* can show a high GI (**Figure 4**). Thus, we recommend caution when considering to use *PAr* as a perfect proxy for GI, as some highly irreplaceable sites could be ignored. We also note that prioritising sites with private alleles means considering the full set of variants occurring in such sites, which, depending on the spatial distribution of alleles and their frequencies, may inflate the proportion of alleles that are

adequately protected overall. Here, we never ended up in protecting less than 80% of the alleles, regardless of the level of protection set prior to the analysis (**Figure 2**).

The correlation between D_A and GI only found in *A. alba* may reflect the peculiar spatial genetic structure observed in this species, where highly differentiated genetic clusters were described as separated by a steep genetic barrier in central Apennines (Piotti et al., 2017). In contrast, low levels of differentiation and extensive gene flow have been described in both *P. heldreichii* populations (Piovesan et al., 2023) and *Q. robur*, particularly across the Po plain in northern Italy (Avanzi et al., 2023). This finding suggests the potential usefulness of relying on both *PAr* and D_A in priority setting when demographic inference supports the occurrence of moderate to high genetic differentiation between sites (e.g., when F_{ST}>0.1). In the presence of high genetic differentiation, sites could be clustered according to their genetic similarity using standard genetic clustering techniques (e.g., Structure, DAPC, Admixture, BAPS) and each genetic cluster could be treated as a distinct conservation feature in SCP with a target of representation (the 'conservation units' approach described in Andrello et al., 2022; Hanson et al., 2020)

4.3. Glacial refugia are priority areas for conservation

The geographic distribution of the large majority of irreplaceable sites for *A. alba* (**Figure 3b**) is in the Apennine mountain range, and included all refugia areas that were proposed for this species based on both genetic and paleobotanical data (Magri et al., 2015; Piotti et al., 2017): the Calabrian arc (southern Apennines) including four sites; the central Apennines, including three sites located across two highly differentiated genetic lineages; and the northern Apennines, including four sites. Despite fragmentation and documented declines in abundance during the Holocene (Magri et al., 2015), such areas still maintain large population sizes, which will likely protect them from future genetic erosion (Forester et al., 2022; Kardos et al., 2021), and show high allelic richness, local adaptations and growth vigour in provenance studies (Kerr et al., 2015; Piotti et al., 2017). Beridze et al. (2023) have also recently shown that sites within refugial areas of *Castanea sativa* deserve high conservation priority. The higher occurrence of sites with high GI in the southern refugial area compared to the northern area (**Figure 3a,b**) reflect high private allelic richness and a long history of genetic isolation from the northernmost populations (Piotti et al., 2017). Physical barriers (i.e., the Gran Sasso and Majella massifs) and possible constraints to long-distance dispersal due to heavy seeds and pollen (Leonarduzzi et al., 2016) may have acted in concert to prevent gene flow from the southern refugial area, facilitating the development of spatial genetic structuring (Piotti et al., 2017).

Priority sites for *Q. robur* generally corresponded to refugial areas, which include the Pre-Alps, the southern slope of the Tuscan-Emilian Apennines, the Tyrrhenian coast and some areas in southern Italy, where the species was likely abundant at the onset of the Holocene (Magri et al., 2015). In particular, irreplaceable sites include six sites along the Po river (northern Italy), five sites in central Italy (two of them in Tuscany) and the putative refugium of 'Bosco Pantano', the southernmost site sampled (**Figure 3f**). Contrary to what is observed in other refugial areas, 'Bosco Pantano' is genetically depauperate, with extremely low allelic richness and effective population size (*Ne*<100) due to recent intensive human impact (Avanzi et al., 2023). The irreplaceability of this site may rather reflect its extremely large genetic distinctiveness and presence of private alleles (Avanzi et al., 2023). Overall, the

identification of priority sites throughout the species range may reflect the occurrence of intense long-distance pollen dispersal diluting genetic structuring over large distances, which is probably the case for both pedunculate oak in northern Italy and Heldreich's pine in its entire Italian distribution (**Figure 3c,d**).

4.4. Practical implications of SCP results

Based on comprehensive genetic datasets characterising neutral genetic diversity in a multi-refugial area, we were able to identify priority sites for conserving IGD in *A. alba*, *P. heldreichii* and *Q. robur*. The applications are manifold.

First, the map of irreplaceable sites (**Figure 3**) can make it possible to assess the current legal level of protection of sites whose elimination would impact the IGD of the species. All *P. heldreichii* sampled populations are within the Pollino National Park, whose perimeter was originally designed to include and protect all Italian populations of the species. The majority of *A. alba* and *Q. robur* populations are within National and Regional Parks, or public Reserves. Nonetheless, some irreplaceable sites for *Q. robur* are in private properties without any protection regime (e.g., the site at highest diversity in Italy; see Avanzi et al., 2023). Our results can contribute to change the protection status of such stands or to raise awareness among privates toward a correct management of forest genetic resources.

Seed collection for *in-situ* and *ex-situ* conservation strategies as well as for reforestation programs, the latter which have been massively funded in Italy in the last years (Mariotti et al., 2022), is usually carried out by the public sector in designated seed stands. Seed stands were not identified on the basis of their genetic characteristics but mostly relying on the knowledge of local experts. Our results can help refine the Italian network of seed stands in terms of optimising the number and geographical position of selected stands. For *A. alba*, for instance, >50 seed stands are registered (MASAF, 2023) but none is present in a relatively small genetic cluster found in central Italy (where two irreplaceable sites were detected) or in the site with the highest genetic diversity in Italy (TDP in Piotti et al., 2017), which we found to be irreplaceable starting from the lowest protection level. For *Q. robur,* there are about 100 seed stands registered, but none of them includes sites in central or southern Italy (the southernmost seed stand is at 43° 21' latitude), where our SCP analysis actually identified five sites with high GI. This means that the genetic diversity of populations at the rear edge of the species distribution, which might have a disproportionate value in fueling the adaptive potential of northernmost populations through gene flow or assisted migration (EUFORGEN, 2021), is currently not represented in seed collection initiatives in Italy. Finally, *P. heldreichii* has no registered seed stands so far, and our findings may be useful in defining an initial priority list.

The Pan-European network of Genetic Conservation Units (GCUs; Lefèvre et al., 2013; http://portal.eufgis.org/) shows a better agreement with our SCP analyses. For instance, the southernmost *Q. robur* population, representing a unique genetic lineage and nowadays composed of only 64 individuals (Avanzi et al., 2023), is recognized as a GCU but not as a seed stand. In addition, despite the absence of seed stands, a GCU for *P. heldreichii* was established in Italy, a 166 ha area within the Pollino National Park that includes five of the irreplaceable sites identified in our analysis. Nonetheless, the selection of GCUs (for which a first, partial genetic characterisation is currently underway in the framework of the EU-funded

FORGENIUS project; www.forgenius.eu) could greatly benefit from spatial conservation prioritisation studies such as the one presented here. Genetic information at the regional level encompassing as much of a species' genetic diversity as possible (especially from marginal populations) and tools for science-based management of GCUs are key prerequisites for efficient monitoring and useful applications of such an infrastructure, as well as for flagging any need for active intervention (EUFORGEN, 2021).

4.5. Conclusions

SCP proved to be an effective method of identifying conservation areas capable of preserving the intraspecific allelic diversity of silver fir, Heldreich's pine and pedunculate oak. In particular, we found that between 50% and 75% of sampled sites are required to protect IGD in these species, which is a higher percentage than the one usually recommended to protect habitats (30%; Convention on Biological Diversity, 2022). Importantly, we found that these percentages hold for both low percentages of alleles to be protected (50%) and more ambitious percentages (>90%). Confirming theoretical expectations (Petit et al., 2003), glacial relics were frequently included in the networks of proposed protected areas. This result may also apply to forest species for which SCP has not yet been undertaken.

Multifaceted applications of data and approaches presented here have been highlighted, with particular reference to optimising the seed collection necessary to fuel ongoing afforestation initiatives such as the commitment for planting at least 3 billion trees in the European Union by 2030 in full respect of ecological principles (EC, 2020). In addition, SCP can provide conservationists with clear guidance on which sites should be prioritised, which is key to the implementation of any action aimed at achieving any conservation target that will be adopted under the CBD (e.g. achieving 90-99% protection of existing forest genetic resources; Convention on Biological Diversity, 2022; Frankham, 2022; Hoban et al., 2023).

Finally, we advocate that future research should focus on (i) describing the relative performance of SCP versus more traditional methods based on allelic diversity in different conservation scenarios, and (ii) the potential impact of different variables on SCP performance (e.g. sample size and set of genetic markers used). In particular, the extension of SCP to genomic data should merit special attention to include both neutral and functional variants in the analysis (Hanson et al., 2020; Nielsen et al., 2023a, 2020; Xuereb et al., 2021), which promises to increase the effectiveness of genetic-informed conservation measures.

Acknowledgments

E. Vajana is supported by the EU-funded project MedForAct (grant agreement ID 101107604). We thank all Parks and Reserves where sampling sites are located for permissions and logistic support. The acknowledgements to specific persons and institutions are reported in the articles where original data were published, indicated in the Materials & Methods section.

Author contributions

Elia Vajana: Conceptualization; Data curation; Formal analysis; Visualization; Writing - original draft. **Marco Andrello**: Conceptualization; Formal analysis; Methodology;

Supervision; Writing - review & editing. **Camilla Avanzi**: Conceptualization; Data curation; Formal analysis; Visualization; Writing - review & editing. **Francesca Bagnoli**: Funding acquisition; Writing - review & editing. **Giovanni G. Vendramin**: Funding acquisition; Supervision; Writing - review & editing. **Andrea Piotti**: Conceptualization; Data curation; Formal analysis; Funding acquisition; Supervision; Visualization; Writing - review & editing.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used *DeepL Write* in order to rephrase specific sentences of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

Alberto, F.J., Aitken, S.N., Alía, R., González- Martínez, S.C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., Savolainen, O., 2013. Potential for evolutionary responses to climate change – evidence from tree populations. Glob. Change Biol. 19, 1645–1661. https://doi.org/10.1111/gcb.12181

Andrello, M., D'Aloia, C., Dalongeville, A., Escalante, M.A., Guerrero, J., Perrier, C., Torres-Florez, J.P., Xuereb, A., Manel, S., 2022. Evolving spatial conservation prioritization with intraspecific genetic data. Trends Ecol. Evol. 37, 553–564. https://doi.org/10.1016/j.tree.2022.03.003

Avanzi, C., Bagnoli, F., Romiti, E., Spanu, I., Tsuda, Y., Vajana, E., Vendramin, G.G., Piotti, A., 2023. The latitudinal trend in genetic diversity and distinctiveness of Quercus robur rear edge forest remnants calls for new conservation priorities. bioRχiv. https://doi.org/10.1101/2023.10.30.564819

Beridze, B., Sękiewicz, K., Walas, Ł., Thomas, P.A., Danelia, I., Fazaliyev, V, Kvartskhava, G., Sós, J., Dering, M., 2023. Biodiversity protection against anthropogenic climate change: Conservation prioritization of *Castanea sativa* in the South Caucasus based on genetic and ecological metrics. Ecol. Evol. 13(5), e10068.

Bonin, A., Nicole, F., Pompanon, F., Miaud, C., Taberlet, P., 2007. Population Adaptive Index: a new method to help measure intraspecific genetic diversity and prioritize populations for conservation. Conserv. Biol. 21, 697–708. https://doi.org/10.1111/j.1523-1739.2007.00685.x

Brewer, S., Cheddadi, R., de Beaulieu, J.L., Reille, M., 2002. The spread of deciduous *Quercus* throughout Europe since the last glacial period. For. Ecol. Manag. 156, 27–48. https://doi.org/10.1016/S0378-1127(01)00646-6

Bucci, G., Vendramin, G.G., Lelli, L., Vicario, F., 1997. Assessing the genetic divergence of *Pinus leucodermis* Ant. endangered populations: use of molecular markers for conservation purposes. Theor. Appl. Genet. 95, 1138–1146. https://doi.org/10.1007/s001220050674

Caudullo, G., Welk, E., San-Miguel-Ayanz, J., 2017. Chorological maps for the main European woody species. Data Brief 12, 662–666. https://doi.org/10.1016/j.dib.2017.05.007

CBD, 2021. First draft of the post-2020 global biodiversity framework.

Convention on Biological Diversity (CBD/COP/DEC/15/4), 2022. Conference of the parties to the Convention of Biologial Diversity (Fifteenth meeting - Part II) (No. Agenda item 9A). Montreal, Canada.

EC, 2020. EU Biodiversity Strategy for 2030. Bringing nature back into our lives, COM(2020) 380 final. Brussels.

El Mousadik, A., Petit, R.J., 1996. High level of genetic differentiation for allelic richness

among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. Theor. Appl. Genet. 92, 832–839.

Erwin, T.L., 1991. An evolutionary basis for conservation strategies. Sci. New Ser. 253, 750–752.

EUFORGEN, 2021. Forest genetic resources strategy for Europe. European Forest Institute.

Exposito-Alonso, M., Booker, T.R., Czech, L., Gillespie, L., Hateley, S., Kyriazis, C.C., Lang, P.L.M., Leventhal, L., Nogues-Bravo, D., Pagowski, V., Ruffley, M., Spence, J.P., Arana, S.E.T., Weiß, C.L., Zess, E., 2022. Genetic diversity loss in the Anthropocene. Science 377, 1431–1435. https://doi.org/10.1126/science.1083264

Fady, B., Cottrell, J., Ackzell, L., Alía, R., Muys, B., Prada, A., González-Martínez, S.C., 2016. Forests and global change: what can genetics contribute to the major forest management and policy challenges of the twenty-first century? Reg. Environ. Change 16, 927–939. https://doi.org/10.1007/s10113-015-0843-9

Fernandez-Fournier, P., Lewthwaite, J.M.M., Mooers, A.Ø., 2021. Do We Need to Identify Adaptive genetic variation when prioritizing populations for conservation? Conserv. Genet. 22, 205–216. https://doi.org/10.1007/s10592-020-01327-w

Forester, B.R., Beever, E.A., Darst, C., Szymanski, J., Funk, W.C., 2022. Linking evolutionary potential to extinction risk: applications and future directions. Front. Ecol. Environ. fee.2552. https://doi.org/10.1002/fee.2552

Frankham, R., 2022. Evaluation of proposed genetic goals and targets for the Convention on Biological Diversity. Conserv. Genet. 23, 865–870. https://doi.org/10.1007/s10592-022-01459-1

Gibbs, J., 1997. Biotic and abiotic factors affecting the dying back of pedunculate oak *Quercus robur* L. Forestry 70, 399–406. https://doi.org/10.1093/forestry/70.4.399

Gougherty, A.V., Keller, S.R., Fitzpatrick, M.C., 2021. Maladaptation, migration and extirpation fuel climate change risk in a forest tree species. Nat. Clim. Change 11, 166–171. https://doi.org/10.1038/s41558-020-00968-6

Gurobi Optimization, LLC, 2023. Gurobi Optimizer Reference Manual.

Hanson, J., Schuster, R., Morrell, N., Strimas-Mackey, M., Edwards, B., Watts, M., Arcese, P., Bennett, J., Possingham, H., 2022. prioritizr: Systematic Conservation Prioritization in R.

Hanson, J.O., Marques, A., Veríssimo, A., Camacho- Sanchez, M., Velo- Antón, G., Martínez- Solano, Í., Carvalho, S.B., 2020. Conservation planning for adaptive and neutral evolutionary processes. J. Appl. Ecol. 57, 2159–2169. https://doi.org/10.1111/1365-2664.13718

Hewitt, G., 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol. J. Linn. Soc. 58, 247–276. https://doi.org/10.1006/bijl.1996.0035

Hoban, S., Bruford, M.W., da Silva, J.M., Funk, W.C., Frankham, R., Gill, M.J., Grueber, C.E., Heuertz, M., Hunter, M.E., Kershaw, F., Lacy, R.C., Lees, C., Lopes-Fernandes, M., MacDonald, A.J., Mastretta-Yanes, A., McGowan, P.J.K., Meek, M.H., Mergeay, J., Millette, K.L., Mittan-Moreau, C.S., Navarro, L.M., O'Brien, D., Ogden, R., Segelbacher, G., Paz-Vinas, I., Vernesi, C., Laikre, L., 2023. Genetic diversity goals and targets have improved, but remain insufficient for clear implementation of the post-2020 global biodiversity framework. Conserv. Genet. https://doi.org/10.1007/s10592-022-01492-0

Hoban, S., Campbell, C.D., da Silva, J.M., Ekblom, R., Funk, W.C., Garner, B.A., Godoy, J.A., Kershaw, F., MacDonald, A.J., Mergeay, J., Minter, M., O'Brien, D., Vinas, I.P., Pearson, S.K., Pérez-Espona, S., Potter, K.M., Russo, I.-R.M., Segelbacher, G., Vernesi, C., Hunter, M.E., 2021. Genetic diversity is considered important but interpreted narrowly in country reports to the Convention on Biological Diversity: Current actions and indicators are insufficient. Biol. Conserv. 261, 109233. https://doi.org/10.1016/j.biocon.2021.109233

Kalinowski, S.T., 2005. hp-rare 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol. Ecol. Notes 5, 187–189.

Kardos, M., Armstrong, E.E., Fitzpatrick, S.W., Hauser, S., Hedrick, P.W., Miller, J.M.,

Tallmon, D.A., Funk, W.C., 2021. The crucial role of genome-wide genetic variation in conservation. Proc. Natl. Acad. Sci. 118, e2104642118. https://doi.org/10.1073/pnas.2104642118

Kerr, G., Stokes, V., Peace, A., Jinks, R., 2015. Effects of provenance on the survival, growth and stem form of European silver fir (*Abies alba* Mill.) in Britain. Eur. J. For. Res. 134, 349–363. https://doi.org/10.1007/s10342-014-0856-9

Lefèvre, F., Alia, R., Bakkebø Fjellstad, K., Graudal, L., Oggioni, S.D., Rusanen, M., Vendramin, G.G., Bozzano, M., 2020. Dynamic conservation and utilization of forest tree genetic resources: indicators for in situ and ex situ genetic conservation and forest reproductive material. European Forest Genetic Resources Programme (EUFORGEN). European Forest Institute.

Lefèvre, F., Koskela, J., Hubert, J., Kraigher, H., Longauer, R., Olrik, D.C., Schüler, S., Bozzano, M., Alizoti, P., Bakys, R., Baldwin, C., Ballian, D., Black- Samuelsson, S., Bednarova, D., Bordács, S., Collin, E., De Cuyper, B., De Vries, S.M.G., Eysteinsson, T., Frýdl, J., Haverkamp, M., Ivankovic, M., Konrad, H., Koziol, C., Maaten, T., Notivol Paino, E., Öztürk, H., Pandeva, I.D., Parnuta, G., Pilipovič, A., Postolache, D., Ryan, C., Steffenrem, A., Varela, M.C., Vessella, F., Volosyanchuk, R.T., Westergren, M., Wolter, F., Yrjänä, L., Zariŋa, I., 2013. Dynamic conservation of forest genetic resources in 33 European countries. Conserv. Biol. 27, 373–384. https://doi.org/10.1111/j.1523-1739.2012.01961.x

Leonarduzzi, C., Piotti, A., Spanu, I., Vendramin, G.G., 2016. Effective gene flow in a historically fragmented area at the southern edge of silver fir (*Abies alba* Mill.) distribution. Tree Genet. Genomes 12, 95. https://doi.org/10.1007/s11295-016-1053-4

López- Cortegano, E., Pérez- Figueroa, A., Caballero, A., 2019. metapop2: Re-implementation of software for the analysis and management of subdivided populations using gene and allelic diversity. Mol. Ecol. Resour. 19, 1095–1100. https://doi.org/10.1111/1755- 0998.13015

López-Cortegano, E., Pouso, R., Labrador, A., Pérez-Figueroa, A., Fernández, J., Caballero, A., 2019. Optimal management of genetic diversity in subdivided populations. Front. Genet. 10, 843. https://doi.org/10.3389/fgene.2019.00843

Magri, D., Agrillo, E., Di Rita, F., Furlanetto, G., Pini, R., Ravazzi, C., Spada, F., 2015. Holocene dynamics of tree taxa populations in Italy. Rev. Palaeobot. Palynol. 218, 267–284. https://doi.org/10.1016/j.revpalbo.2014.08.012

Maiorano, L., Cheddadi, R., Zimmermann, N.E., Pellissier, L., Petitpierre, B., Pottier, J., Laborde, H., Hurdu, B.I., Pearman, P.B., Psomas, A., Singarayer, J.S., Broennimann, O., Vittoz, P., Dubuis, A., Edwards, M.E., Binney, H.A., Guisan, A., 2013. Building the niche through time: using 13,000 years of data to predict the effects of climate change on three tree species in Europe. Glob. Ecol. Biogeogr. 22, 302–317. https://doi.org/10.1111/j.1466-8238.2012.00767.x

Mariotti, B., Mezzalira, G., Allasia, E., Fazio, F., Fiorentin, R., Maltoni, A., Marchetti, M., Matteucci, G., Mori, P., Motta, R., Piotti, A., Rositi, A., Sabatti, M., Tognetti, R., Salbitano, F., 2022. The Italian forest nursery sector at the crossroads: challenges and strategies. For. - Riv. Selvic. Ed Ecol. For. 19, 85–94. https://doi.org/10.3832/efor4193-019

Marris, E., 2007. Conservation priorities: What to let go. Nature 450, 152–155.

MASAF, 2023. *Cartografia dei materiali di base per la produzione di materiali forestali di moltiplicazione*.

Moilanen, A., Possingham, H.P., Polasky, S., 2009. A mathematical classification of conservation prioritization problems, in: Moilanen, A., Wilson, K.A., Possingham, H.P. (Eds.), Spatial Conservation Prioritization: Quantitative Methods and Computational Tools. Oxford University Press, Oxford, UK.

Naidoo, R., Balmford, A., Ferraro, P., Polasky, S., Ricketts, T., Rouget, M., 2006. Integrating economic costs into conservation planning. Trends Ecol. Evol. 21, 681–687. https://doi.org/10.1016/j.tree.2006.10.003

Nielsen, E.S., Beger, M., Henriques, R., Von Der Heyden, S., 2023a. Integrating environmental, evolutionary, and socioeconomic vulnerability to future-proof coastal conservation planning. Biol. Conserv. 286, 110302. https://doi.org/10.1016/j.biocon.2023.110302

Nielsen, E.S., Beger, M., Henriques, R., Von Der Heyden, S., 2020. A comparison of genetic and genomic approaches to represent evolutionary potential in conservation planning. Biol. Conserv. 251, 108770. https://doi.org/10.1016/j.biocon.2020.108770

Nielsen, E.S., Hanson, J.O., Carvalho, S.B., Beger, M., Henriques, R., Kershaw, F., Von Der Heyden, S., 2023b. Molecular ecology meets systematic conservation planning. Trends Ecol. Evol. 38, 143–155. https://doi.org/10.1016/j.tree.2022.09.006

Panagos, P., Borrelli, P., Poesen, J., Ballabio, C., Lugato, E., Meusburger, K., Montanarella, L., Alewell, C., 2015. The new assessment of soil loss by water erosion in Europe. Environ. Sci. Policy 54, 438–447. https://doi.org/10.1016/j.envsci.2015.08.012

Paz-Vinas, I., Loot, G., Hermoso, V., Veyssière, C., Poulet, N., Grenouillet, G., Blanchet, S., 2018. Systematic conservation planning for intraspecific genetic diversity Proceedings of the Royal Society B, 20172746. http://dx.doi.org/10.1098/rspb.2017.2746

Petit, R.J., Abdelhamid, E.M., Odile, P., 1998. Identifying populations for conservation on the basis of genetic markers. Conservation biology 12, 844–855.

Petit, R.J., Aguinagalde, I., de Beaulieu, J.-L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Müller-Starck, G., Demesure-Musch, B., Palme, A., Martín, J.P., Rendell, S., Vendramin, G.G., 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. Science 300, 1563–1565. https://doi.org/10.1126/science.1083264

Petit, R.J., Brewer, S., Bordács, S., Burg, K., Cheddadi, R., Coart, E., Cottrell, J., Csaikl, U.M., van Dam, B., Deans, J.D., Espinel, S., Fineschi, S., Finkeldey, R., Glaz, I., Goicoechea, P.G., Jensen, J.S., König, A.O., Lowe, A.J., Madsen, S.F., Mátyás, G., Munro, R.C., Popescu, F., Slade, D., Tabbener, H., de Vries, S.G.M., Ziegenhagen, B., de Beaulieu, J.-L., Kremer, A., 2002. Identification of refugia and post-glacial colonisation routes of European white oaks based on chloroplast DNA and fossil pollen evidence. For. Ecol. Manag. 156, 49–74. https://doi.org/10.1016/S0378-1127(01)00634-X

Petit, R.J., Hampe, A., Cheddadi, R., 2005. Climate changes and tree phylogeography in the Mediterranean. Taxon 54, 877. https://doi.org/10.2307/25065474

Piotti, A., Leonarduzzi, C., Postolache, D., Bagnoli, F., Spanu, I., Brousseau, L., Urbinati, C., Leonardi, S., Vendramin, G.G., 2017. Unexpected scenarios from Mediterranean refugial areas: disentangling complex demographic dynamics along the Apennine distribution of silver fir. J. Biogeogr. https://doi.org/10.1111/jbi.13011

Piovesan, G., Rita, A., Biondi, F., Baliva, M., Borghetti, M., Brunetti, M., De Vivo, G., Di Filippo, A., Dinella, A., Gentilesca, T., Maugeri, M., Palli, J., Piotti, A., Saba, E.P., Ripullone, F., Schettino, A., Vendramin, G.G., 2023. Bell-shaped tree-ring responses to air temperature drive productivity trends in long-lived mountain Mediterranean pines. Sci. Total Environ. 890, 164103. https://doi.org/10.1016/j.scitotenv.2023.164103

Pressey, R.L., Johnson, I.R., Wilson, P.D., 1994. Shades of irreplaceability: towards a measure of the contribution of sites to a reservation goal. Biodivers. Conserv. 3, 242–262. https://doi.org/10.1007/BF00055941

R Core Team, 2022. R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna Austria.

Robuchon, M., Pavoine, S., Véron, S., Delli, G., Faith, D.P., Mandrici, A., Pellens, R., Dubois, G., Leroy, B., 2021. Revisiting species and areas of interest for conserving global mammalian phylogenetic diversity. Nat. Commun. 12, 3694. https://doi.org/10.1038/s41467-021-23861-y

Robuchon, M., Silva, J. da, Dubois, G., Gumbs, R., Hoban, S., Laikre, L., Owen, N.R., Perino, A., 2022. Conserving species' evolutionary potential and history:

opportunities under the new post-2020 global biodiversity framework (preprint). Preprints. https://doi.org/10.22541/au.166568699.98478640/v1

Ruosch, M., Spahni, R., Joos, F., Henne, P.D., Van Der Knaap, W.O., Tinner, W., 2016. Past and future evolution of *Abies alba* forests in Europe – comparison of a dynamic vegetation model with palaeo data and observations. Glob. Change Biol. 22, 727–740. https://doi.org/10.1111/gcb.13075

Santini, F., Andrisano, T., Leonardi, S., Ciaschetti, G., Labriola, M., Vendramin, G.G., Piotti, A., 2018. Tracking the origin of silver fir plantations along the boundary between different genetic clusters in central Apennines: Implications for their management. For. Ecol. Manag. 408, 220–227. https://doi.org/10.1016/j.foreco.2017.10.045

Schweitzer, J.A., Fischer, D.G., Rehill, B.J., Wooley, S.C., Woolbright, S.A., Lindroth, R.L., Whitham, T.G., Zak, D.R., Hart, S.C., 2011. Forest gene diversity is correlated with the composition and function of soil microbial communities. Popul. Ecol. 53, 35–46. https://doi.org/10.1007/s10144-010-0252-3

Tapio, I., VäRv, S., Bennewitz, J., Maleviciute, J., Fimland, E., Grislis, Z., Meuwissen, T.H.E., Miceikiene, I., Olsaker, I., Viinalass, H., Vilkki, J., Kantanen, J., 2006. Prioritization for conservation of northern European cattle breeds based on analysis of microsatellite data. Conserv. Biol. 20, 1768–1779. https://doi.org/10.1111/j.1523-1739.2006.00488.x

Thomas, F.M., Blank, R., Hartmann, G., 2002. Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. For. Pathol. 32, 277–307. https://doi.org/10.1046/j.1439-0329.2002.00291.x

Vane-Wright, R.I., Humphries, C.J., Williams, P.H., 1991. What to protect?—Systematics and the agony of choice. Biol. Conserv. 55, 235–254. https://doi.org/10.1016/0006-3207(91)90030-D

von Takach, B., Penton, C.E., Murphy, B.P., Radford, I.J., Davies, H.F., Hill, B.M., Banks, S.C., 2021. Population genomics and conservation management of a declining tropical rodent. Heredity 126, 763–775. https://doi.org/10.1038/s41437-021-00418-9

Von Takach, B., Sargent, H., Penton, C.E., Rick, K., Murphy, B.P., Neave, G., Davies, H.F., Hill, B.M., Banks, S.C., 2023. Population genomics and conservation management of the threatened black-footed tree-rat (*Mesembriomys gouldii*) in northern Australia. Heredity 130, 278–288. https://doi.org/10.1038/s41437-023-00601-0

Weitzman, M.L., 1998. The Noah's ark problem. Econometrica 66, 1279–1298.

Weitzman, M.L., 1993. What to preserve? An application of diversity theory to crane conservation. Q. J. Econ. 108, 157–183.

Wilson, K.A., Cabeza, M., Klein, C.J., 2009. Fundamental concepts of spatial conservation prioritization, in: Moilanen, A., Wilson, K.A., Possingham, H.P. (Eds.), Spatial Conservation Prioritization: Quantitative Methods and Computational Tools. Oxford University Press, Oxford, UK.

Xuereb, A., D'Aloia, C.C., Andrello, M., Bernatchez, L., Fortin, M., 2021. Incorporating putatively neutral and adaptive genomic data into marine conservation planning. Conserv. Biol. 35, 909–920. https://doi.org/10.1111/cobi.13609