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Effect of number of seed parents on the genetic diversity of artificially regenerated stands of holm oak

Abstract

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Artificial populations of holm oak (*Quercus ilex* L. s. l.) and their natural seed origin populations selected from Andalusia (Southern Spain) and Sicily (Southern Italy) were analysed with six nuclear microsatellite loci to explore the effect of seed collection methods. Low effective allele numbers (3.07-6.67) and extremely low effective population sizes (35.4-102.9) were detected in both reforestation stands, probably due to an inappropriate forester seed collection strategy limited to a few mother trees. Analysis of simulated progenies showed that optimal sampling size to obtain seed collections genetically similar to the origin population should range from 20 to 50 individuals, but could be different between the two regions, being higher in the more genetically diverse Andalusia.

Introduction

The reforestation with autochthonous species should preserve genetic diversity to maintain fitness and adaptability potential of forest ecosystems. The sampling strategy, stand structure and mating system of a tree population determine the genetic structure of the harvested material and, consequently, the similarity of the seedlots to the origin population (Degen & al. 1996).

Within the silvicultural framework, the relationship between level of genetic diversity and population size has frequently been analysed with reference to adult trees (see Young & Boyle 2000). Another approach proposes expressing the diversity of seedlots in terms of number of seed parents included in the harvest (eg. Lexer & al. 1999), shifting the point of view to the progeny (i.e. seeds as forest reproductive material). Recently, Blakesley & al. (2004) addressed the question of the minimum number of mother trees required to maintain the original genetic variability in the seedlots collected for artificial regeneration of an endangered tree species.

In the present contribution, the effects of the seed sampling strategy on the genetic diversity of simulated seedlot samples have been investigated, and the results have been compared with two empirical cases of artificial regeneration with *Quercus ilex* L. s. l., located in Sicily (Southern Italy) and in Andalusia (Southern Spain).

Materials and methods

The natural and artificial populations for sampling were selected taking into account the informations supplied by forestry agents and nursery workers to meet the following criteria: 'natural seed origin population', a population usually used as source of seed for forestry nurseries; 'nursery', a stock of seedlings (sampled only in Sicily) whose seed-source is the above mentioned natural population; 'artificially regenerated population', a reforested stand whose seed-source is the above-mentioned seed origin population. The Sicilian origin population, Piano Zucchi forest, and nursery, Piano Noce, are located in the Madonie mountain chain (east of the province of Palermo), while the regenerated stand is situated in the Palmeto mountain (west of the same province). In Andalusia, the seed origin population is an open formation (*dehesa*), El Cazador, in the Sierra Morena (Sevilla), while the artificial stand is located in the Sierra de Gador (Almería). The leaves from 30-40 individuals were collected per sample. The individuals were sampled in natural populations maintaining an average distance of 50 m between them.

Six nuclear microsatellite loci were used for genetic analysis: MSQ4, QpAG15, QpAG36, QpAG46 transferred to *Q. ilex* by Soto & al. (2003); QrZAG11 and QrZAG20 modified from Kampfer & al. (1998).

To understand the effect of the number of seed parents on the genetic diversity of seedlot samples, computer simulations were run to reproduce the biological conditions of the natural systems studied. Two stands, of 1000 differently aged individuals each, were simulated on the basis of the allelic frequencies of the natural seed origin populations. The *in silica* Sicilian stand had a density of 30 trees/ha, while the Andalusian stand had 60 trees/ha. Fictitious seed collections (1000 seeds) from different numbers of randomly selected trees (2, 10, 20, 30, 50, 100) were produced.

The computer simulations were performed with ECO-GENE (Degen & al. 1996). The effective number of alleles of simulated seedlots was calculated as $A_e = 1/\sum p^2$ (where p = allele frequency) with POPGENE 1.31 (Yeh & al. 1999). Effective population size of sampled populations (N_e) was estimated with a likelihood procedure which takes into account the changes in allelic frequencies between two generations and the possibility of migration with MLNE (Wang & Whitlock 2003).

Results and discussion

For the Sicilian populations 120 individuals were analyzed and 65 alleles were found, while the Andalusian populations comprised 157 individuals with a total allele number of 90.

As expected, the effective number of alleles (A_e) of fictitious seedlots increased with the number of seed parents, because of progressively higher effective population size (Fig. 1a, b). The regression curve reached a *plateau*, corresponding approximately to the A_e value of the fictitious seed origin population. In the Sicilian case (Fig. 1a) A_e of simulated progenies reached that of the origin population with 10 mother trees, while 20 mother trees were needed in the Andalusian simulation. A_e absolute values in the Andalusian case were also notably higher when compared with Sicilian A_e values (Fig. 1b). This difference is explained by the higher genetic richness found in the Andalusian sampled populations

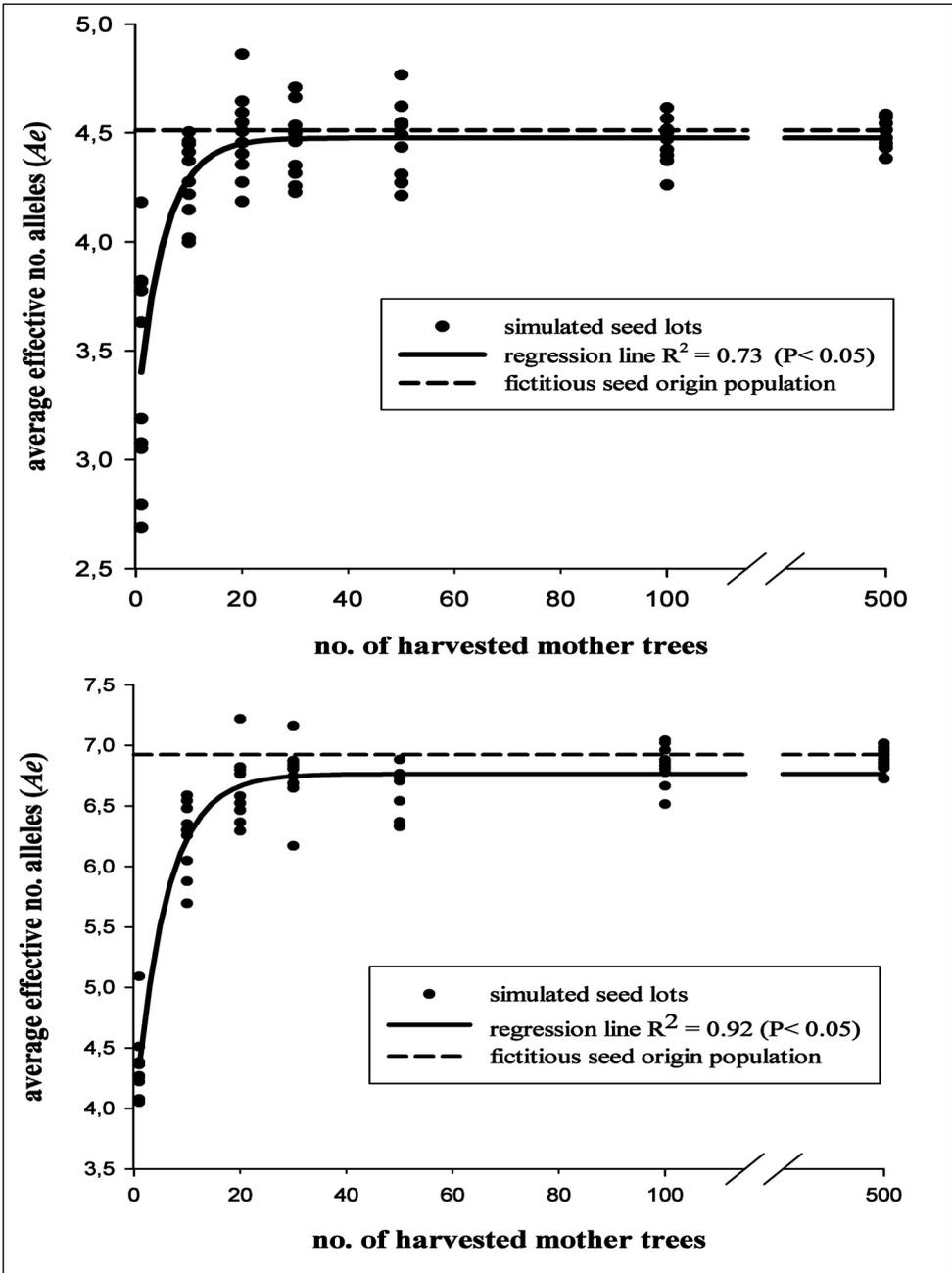


Fig. 1. Effect of number of seed parents on the effective allele number (mean value) of simulated seedlot samples (dots) harvested from a fictitious seed origin population (dashed line). For each number of seed parents (2, 10, 20, 30, 50, 100, 500) 10 simulations were performed. *Ae* of fictitious seedlots increases with the number of seed parents because of progressively higher effective population size, until reaching a *plateau*. a) Sicily, b) Andalusia.

and suggests that the number of mother trees required to maintain the most common alleles of the origin population in a harvested seedlot is smaller in Sicily than in Andalusia.

Comparing the results of the theoretical model with the empirical estimates of A_e corresponding to the sampled artificially regenerated stands, the value of Monte Palmeto ($A_e = 3.07$) might indicate a number of mother trees lower than 10 (the same could be concluded for the nursery A_e value, $A_e = 3.65$), while the Andalusian artificial stand ($A_e = 6.67$) might indicate a number of mothers between at least 10 and 20. Estimates of effective population sizes (N_e) are congruent with those results, with $N_e = 52.5$ for the nursery and $N_e = 35.4$ for the reforestation in Sicily; and $N_e = 102.9$ for the reforestation in Andalusia. These values are very low considering that N_e generations would reduce the heterozygosity due to genetic drift by 50%. In both cases examined, the results observed can be explained by a seed harvest from a limited number of trees, whose consequences on the diversity of the progenies are similar to those due to a bottleneck or founder effect.

Conclusions

Although the studied populations of Sicily and Andalusia share the same trend of variation for the effective number of alleles of seedlots in dependence on the number of harvested trees, optimal sampling size to obtain seed collections genetically similar to the origin population could be different for the two regions, being higher in Andalusia. As a guideline, this study suggests that the number of sampled trees should range from 20 to 50 individuals. The only reference on forest management available at the moment is the Austrian forest seed law, which fixes the minimum number of seed parents per stand at 20 in the case of the oaks *Quercus petraea* and *Q. robur* (Lexer & al. 1999).

Since extremely low female and total effective population sizes were detected, the studied reforestation stands seem to have been subject to a genetic process whose natural equivalent is a bottleneck or founder effect. This is probably the effect of an inappropriate forester seed collection strategy limited to a few trees that might be accentuated by mating system properties (differences in flowering phenology, annual variation in fertility, stand structure and density), causing some genetic differences among harvested seedlots.

The results of this study highlight the importance of taking into account differences in forest genetic resources among regions which might require specific management practices according to the characteristics of each particular species in each area.

Finally, nuclear microsatellites have proven to be useful tools for investigating genetic diversity of nursery seedlots and artificially regenerated stands of holm oak, including cases when genotype information of parent identity is lacking.

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