

Allocating individuals to avoid inbreeding in *ex situ* conservation plantations: so far, so good

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Abstract The only cost-effective way to control inbreeding in *ex situ* forest tree plantations is often to allocate trees in such a way that the possibility of close relatives mating is small and, consequently, inbreeding does not increase too much over time. The classical *permutated neighbourhood* methods look for the configuration in which no ramets of the same genet are planted in the surroundings (neighbourhoods) of a particular tree but deny the influence of more distant trees. Another limitation of these methods is that they cannot incorporate any other genetic (e.g. kinship) or ecological (e.g. phenology) information. We have developed a new method based on the minimisation of the global probability of generating inbred offspring for the whole population. Improvements of this method from the classical ones are: (i) it takes into account all the trees (whether near or far) and not only the neighbours; (ii) different pollen dispersal functions can be implemented, fitting the particularities of each species and population; (iii) it allows for the integration of all available information about the genetic relationship between trees; and (iv) it is flexible allowing for particular crosses to be banned or encouraged. The novel method showed a better performance than classical ones both for simulated data and a case study under a broad range of scenarios. Magnitude of the benefit depends on the actual and assumed parameters for the pollen dispersal function and the

relationship between trees, but even in the simple case where only clone identity is considered some advantage can be obtained by implementing the new algorithm.

Keywords *Ex situ* plantations · Seed orchards · Forest trees · *Pinus pinaster* · Kinship

Introduction

Conservation efforts in forest trees have been centred on *in situ* approaches, where populations of interest are preserved in their natural environments (Rogers 2004; Vaxevanidou et al. 2006). The *in situ* approach is appealing because it respects the natural dynamics and evolution of the populations and can, at the same time, preserve also a wide-range of organisms associated to the target species and their complex ecological interactions. However, *in situ* conservation has necessarily to be complemented with *ex situ* approaches (in the case of forest trees generally involving conservation plantations or seed banks) when the resources to preserve are threatened by local factors such as forest fires, overgrazing by cattle, diseases and pests, hybridization with related species or human-induced ecological changes, in particular the current process of rapid climatic change (Colas et al. 1997; Frankham et al. 2002; Segarra-Moragués et al. 2005).

For example, the native population of Monterey pine (*Pinus radiata* D. Don, one of the most important sources of softwood in the world, with more than 5 million ha of commercial plantations) from Guadalupe Island (Mexico) comprised only ~220 overmature individuals in 2001, about half the census number reported in 1964 (Bannister 1965; Vargas-Hernández et al. 2004). Insular populations of Monterey pine present singular adaptations and carry

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resistance to important diseases affecting mainland populations (Ledig et al. 1998; Rogers 2002; and references therein), and are valuable resources for conservation and breeding. Grazing of natural regeneration by goats and severe environmental conditions seem the main causes of decline in this highly endangered population and ex situ conservation measures have been found to be inexcusable (Ledig et al. 1998). Another seminal example is that of Catalina mahogany (*Cercocarpus traskiae*), a narrow-endemic small tree confined to south-western Santa Catalina Island (California). In this case, threats from grazing and rooting by wild and domestic animals are accompanied by gene pool erosion through introgression with a related species, *C. betuloides* (Rieseberg et al. 1989; Rieseberg and Swensen 1996). Transplantation of cuttings from the remaining individuals of *C. traskiae* to other parts of the island where hybridization risks were negligible is thus recommended.

It has been known for a long time the existence of lethal or deleterious effects in offspring produced by mating of individuals related by ancestry (i.e. inbreeding depression), which often are associated with reduced reproductive fitness (Young et al. 2000; Frankham et al. 2002). Inbreeding is unavoidable in small isolated populations, as all individuals will be related by coancestry over time, a fact that is exacerbated by fine-scale spatial genetic structure and restricted dispersal. Many temperate forest trees lack prezygotic self-incompatibility systems (e.g. *Pinus* spp.), although they retain considerable heterozygosity because of early inbreeding depression. For instance, pines, a widespread genus comprising over 100 species, typically have a high number of lethal equivalents (nine in *Pinus sylvestris*; Savolainen et al. 1992) and suffer strong inbreeding depression (Williams and Savolainen 1996; Durel et al. 1996), which results in the purge of inbred individuals at early stages of development (Muona et al. 1987; Morgante et al. 1993).

From the above it is easy to see that avoiding mating among relatives in the management of forest tree populations is especially important. Different solutions have been developed to avoid mating among relatives in conservation or breeding programs. Among them, directed mating schemes avoiding to a certain degree consanguineous matings are probably the more suitable to accomplish both conservation and breeding goals (e.g. *MAI*, Wright 1921; *circular mating*, Kimura and Crow 1963; *compensatory mating*, Caballero et al. 1996; *mcm*, Fernández and Caballero 2001). However, in forest trees directed mating schemes are rarely used, given the high level of management required, and are generally restricted to breeding programs of commercial species where conservation goals are only secondary. Managers of open-pollinated tree plantations usually tend to avoid mating among relatives

through physical isolation (e.g. using “barriers” of other species) or, more commonly, by using plantation designs that separate like-individuals in space. Indeed, the larger the distance that separates two trees, the less likely they mate (see reviews in Adams and Burczyk 2000; Smouse and Sork 2004; Sork and Smouse 2006). In addition, some tree plantations managed for quality seed production (i.e. seed orchards) incorporate management criteria, such as fertility control, selective thinning or selective harvest, aiming at reducing inbreeding in seed crops (Kang and Lindgren 1999; Kang et al. 2001).

Traditionally, plantations designed to avoid mating among relatives have relied in (i) *random* designs, (ii) *incomplete block* designs or (iii) *permuted neighbourhood* and related methods (e.g. La Bastide 1967; Bell and Fletcher 1978; Chakravarty and Bagchi 1993; Nester 1994). *Permuted neighbourhood* approaches have been shown to be superior to the others (Vanclay 1991). These methods allocate individuals avoiding ramets of the same clone in neighbouring positions. For instance, in the popular implementation of Bell and Fletcher (1978) (the COOL software), the number of rows of neighbours that separate two ramets of the same clone can vary from 1 to 8, depending on the user requirements. *Permuted neighbourhood* methods have been extensively applied to seed orchard and conservation plantation designs till nowadays.

However, these methods have several limitations. As their name suggests, the allocation algorithm only considers the closest trees, which is not adequate for most forest species because they often fit highly leptokurtic (i.e. fat-tailed) pollen dispersal kernels (e.g. *Pinus sylvestris*, Robledo-Arnuncio and Gil 2005; *Pinus pinaster*, Lucas et al. 2008; *Sorbus torminalis*, Oddou-Muratorio et al. 2005). A further limitation of *permuted neighbourhood* methods is that they do not allow for the incorporation of any information about the relationship between individuals (apart from the fact of belonging to the same or different clones; but see Vanclay 1991), which might be a severe limitation in ex situ conservation plantations, in particular when considering the high levels of inbreeding and coancestry usually found in natural endangered populations. Finally, current implementations of *permuted neighbourhood* methods are neither flexible nor user-friendly, which has limited their use and makes difficult to accommodate different management conditions and species particularities.

In natural populations of forest trees, genealogies are usually lacking (at least for the paternal lineage) and, therefore, it is not possible to construct a pedigree-based coancestry matrix. However, the recent development of DNA screening techniques have provided with an increasingly large number of molecular markers that can be used to estimate the genetic relationships between

individuals. Different methods have been developed to infer the coancestry between pairs of individuals (see, for example, Toro et al. 2002; Oliehoek et al. 2006) or for the whole population at a time (Butler et al. 2004, Fernández and Toro 2006). A revision of advantages and limitations of each method can be found, for example, in Fernández and Toro (2006).

Our goal here is to develop a method, based on mathematical optimisation, to allocate trees in ex situ conservation plantations and seed orchards avoiding as much as possible the increase of inbreeding in the following generation (with no management). This method provides a global solution (i.e. that considers all trees in the plantation) and is flexible, being able to include different pollen dispersion functions and taking into account any available information about the genetic relationships among trees. Our new-developed method has immediate applications in breeding, quality seed production and, very particularly, in the establishment of forest tree conservation plantations.

Materials and methods

Algorithm

In an open-pollinated tree plantation, the probability of a mother-tree of generating inbred offspring depends on its coancestry with any other tree (assuming monoecious species with weak incompatibility systems, such as many temperate forest trees) and the probability of being pollinated by each of the candidate father trees. Therefore, the optimum strategy to allocate N trees in a conservation plantation is to minimise the global probability across the whole population of generating inbred offspring, expressed by the function:

$$\sum_{x=1}^N \frac{\sum_{y=1}^N p(x,y)f(x,y)}{\sum_{y=1}^N p(x,y)} \quad (1)$$

where $p(x, y)$ is the probability for a tree x of being pollinated by another tree y , and $f(x, y)$ is the coancestry between these trees. Note that probabilities have to be standardised for each tree to ensure the sum of probabilities equals one. When dealing with monoecious species both the probability associated to the couple (x, y) and the reciprocal combination (y, x) have to be considered, as both trees may serve as mother tree. The probability of a tree to be fertilised by pollen produced by itself, i.e. the self-fertilisation rate, is represented by $x = y, p(x, x)$.

For two different trees (i.e. two different locations), $p(x, y)$ is inversely related to the distance between them $[d(x, y)]$, because the larger the distance, the lower the probability of pollen reaching the target position. In the common case in ex situ conservation plantations of an orthogonal distribution of trees with rows $i = 1, \dots, R$ and columns $j = 1, \dots, C$, the distance (in Euclidean terms) between trees located at positions ij and kl is $d(ij, kl) = \sqrt{(m \cdot (i - k))^2 + (m \cdot (j - l))^2}$, where m is the distance between adjacent rows (columns). Therefore, formula (1) can be rewritten as:

$$\sum_{i=1}^R \sum_{j=1}^C \sum_{k=1}^R \sum_{l=1}^C p(ij, kl)f(ij, kl) \quad (2)$$

being each pair of row and column the coordinates of possible locations for a tree. An optimal implementation of the algorithm should be flexible and allow for irregular plantation designs, in order to account for the particularities of the plantation sites.

From the statements above it is clear to see that the problem of avoiding the increase of inbreeding in the following generation for an open-pollinated plantation reduces to finding the optimal place for each tree in order to minimise the value of formula (1).

Coancestry matrix

To estimate the global probability of producing inbred progeny, the algorithm uses all the available information about individuals' relationships via a pairwise coancestry matrix. The coancestry coefficient, as defined by Malècot (1948), is the probability of sampling two alleles identical by descent (IBS) taken at random one from each individual in the same locus. In the common case that only information about the clone to which the ramets belong is available, it is possible to use a binary matrix where 0.5 denote pairs of trees of the same clone and zero is given for the rest of the comparisons. Previous algorithms were only able to deal with this kind of information (see, for instance, Bell and Fletcher 1978; Vanclay 1991).

The full power of our algorithm is expressed when a coancestry matrix (whatever calculated from the pedigree or computed from molecular marker assays) is available. Mixed methods, where some particular crosses are avoided (e.g. those between individuals from the same clone or particular unwanted combinations) or promoted (e.g. pairs of trees with good specific combination ability or with traits that would potentially increase offspring fitness) while others depend only on pairwise coancestry, can also be implemented.

Pollen dispersal kernels

As previously stated, the probability of mating is expected to decrease with distance between trees (see reviews in Adams and Burczyk 2000; Smouse and Sork 2004; Sork and Smouse 2006). But the particular rate depends on the pollen dispersal probability function of the species under study. Our algorithm can accommodate different dispersal probability functions (i.e. dispersal kernels) to account for this fact. Here, we will develop the method for two widely used pollen dispersal kernels (actually, linear and power-exponential ones), although any other can easily be implemented.

Linear

Under this dispersal function, probabilities can be calculated as $p(x, y) = a + b \cdot d(x, y)$, where a is the intercept (i.e. the probability of being pollinated by a tree in the same position as the target tree, not the self-pollination rate), b is the slope of the linear function and $d(x, y)$ is the distance between trees. Consequently, this model assumes that the probability of receiving pollen from a tree located further than a threshold distance (actually $-a/b$) is null.

Power-exponential

The expression for calculating the pollination probability in this case is

$$p(x, y) = \frac{\beta}{2\pi\alpha^2\Gamma(2/\beta)} \exp\left(-\left(\frac{d(x, y)}{\alpha}\right)^\beta\right),$$

where α and β are the scale and shape parameters of the distribution, respectively, $d(x, y)$ is the distance between trees and Γ is the gamma function (Tufto et al. 1997; Clark et al. 1998). Under this probability distribution, the mean pollination distance is $\delta = \alpha[\Gamma(3/\beta)/\Gamma(2/\beta)]$, being α and β the parameters of the power-exponential dispersal kernel and Γ the gamma function.

The power-exponential curve family is widely used in dispersal studies due to its ability to accommodate popular functions such as the exponential ($\beta = 1$) and the normal ($\beta = 2$) distributions. The power-exponential general function can also represent more leptokurtic kernels ($\beta < 1$). Recent experimental data has shown that leptokurtic kernels (i.e. kernels with fat-tails) are adequate for modelling pollen dispersal in forest trees (Austerlitz et al. 2004; Robledo-Arnuncio and Gil 2005).

Optimisation method

The method is based on a *simulated annealing* algorithm (Kirkpatrick et al. 1983) to search across the feasible space of plantation designs. The method starts from a random solution, obtained by randomly assigning a free spatial position to every tree in the population. From this starting point, alternative solutions are generated by exchanging the positions of two randomly chosen trees. Then the ‘values’ of the present and the alternative solution (i.e. the probability of generating inbred offspring calculated from the two considered plantation designs) are calculated. Acceptance of the alternative solution occurs with a probability $\Omega = \exp(-\Delta/T)$, where Δ is the difference between the values of the alternative and present solutions and T is a ‘cooling’ factor or temperature. The order of the subtraction should be (alternative – present) as the *simulated annealing* algorithm is a minimization method. Obviously, if $\Delta < 0$ then $\Omega > 1$ and the alternative solution is, therefore, accepted. A large number of alternative solutions are generated and tested and, then, the value of T is reduced by a factor Z . Another series of solutions is generated, the parameter T reduced and so on. At the beginning of the process many alternative solutions are accepted, but as T decreases it becomes more difficult to accept new solutions, unless they are better than the present one. The optimal solution is assumed to be found either (i) when no alternative solution is accepted for all modifications generated at a given ‘temperature’ (since the solution is then very unlikely to be improved at either the current or lower temperatures), or (ii) when the maximum number of steps are performed.

In the first step, alternative solutions are constructed by exchanging the positions of many couples of trees at a time (actually 10) in order to perform a broad search across the space of solutions. But as the process advances, smaller modifications are generated to allow for a fine searching in a particular area. The number of trees changed per solution is automatically decided by the algorithm and depends on the number of rejections/acceptances along the previous step. Actually, this number is 10-multiplied by the proportion of alternative solutions accepted in the previous step.

In the particular implementation presented, up to 400 steps (i.e. different values for T) with 1,000 alternative solutions each were allowed. These figures, as well as the rate of decrease of the T cooling factor, Z , were set based on previous simulations performed to ‘tune’ the algorithm. Too rapid decrease of T leads the algorithm to get stuck in non-optimal solutions, while too slow reduction is a waste of computing time. The training period for the algorithm allowed for finding an equilibrium value in $Z = 0.9$.

Simulated data

To test its efficiency and to show some of the basic advantages of the proposed algorithm against traditional strategies, the method was first implemented on two simplified simulated situations.

Scenario 1

The task was allocating 16 repeats of 16 genets in a 16×16 grid. The only known relationship between trees was belonging or not to the same genet. A power exponential pollen dispersal kernel was assumed with several combinations of parameters: $\alpha = 4.3$ with $\beta = 2$ or $\beta = 1$, and $\alpha = 24.08$ with $\beta = 0.67$ or $\beta = 0.2$.

Scenario 2

The problem consisted in allocating 100 trees in an orthogonal plantation of 10 rows and 10 columns with 1 m of distance between them. Trees belonged to 10 different clones (numbered from 1 to 10) with 10 ramets each. Clones were unrelated except for clone 1 and 2 that arose from two full-sibs. Therefore, values of the coancestry matrix were 0.5 for pairs of individuals of the same clone, 0.25 for positions corresponding to one ramet of clone 1 and another from clone 2, and zeros for the rest of relations. In this case only the exponential pollen dispersal kernel with parameters $\alpha = 24.08$ and $\beta = 0.67$ (see Robledo-Arnuncio and Gil 2005) was tested.

Case study

Maritime pine (*Pinus pinaster* Aiton) is a widespread Mediterranean pine with both commercial and ecological importance. Ex situ plantations of this species usually have the double objective of conservation of valuable genetic resources and production of high quality seed for reforestation. A maritime pine conservation/seed production plantation was established in 1993 in Valsaín (Segovia), considering only clone identity and established following a *permuted neighbourhood* design (see Bell and Fletcher 1978 for details on the design). Population was formed by a total of 1091 maritime pine individuals belonging to 61 genets (3–27 ramets per genet). Plantation site had 27 rows \times 51 columns with some banned positions because of unsuitable terrain. Distance between adjacent rows/columns was 5 m. In the realised design of the plantation, up to three ‘neighbourhoods’ were avoided for trees of the same genet, but other numbers (1–8) were considered in

this study to further investigate the performance of this classical method.

Pollen dispersal kernels

To model pollen dispersal, we used both power-exponential and linear functions. For power exponential dispersal kernels, we first used parameter values for *Pinus sylvestris* reported in the literature ($\alpha = 24.08$ and $\beta = 0.67$; see Robledo-Arnuncio and Gil 2005), which are very similar to those obtained from other pine species (see, for instance, Lucas et al. 2008), yielding an average dispersal distance of 137.48 m. Then, to simulate the effect of more leptokurtic functions (which often, although not always, result on larger average dispersal distances), we assumed a $\beta = 0.20$, yielding in this case an average dispersal distance of nearly 6,000 km, which can be considered an approximation to random mating (i.e. close to independence of spatial structure). To explore a wider range of scenarios, higher values of β were also tested (in particular $\beta = 1$ and $\beta = 2$, corresponding to exponential and normal distributions, respectively). In the latter situations, α was set to 4.3 in order to obtain tractable average dispersal distances (8.6 m for $\beta = 1$ and 3.8 m for $\beta = 2$).

The values of parameters a and b of the linear function were calculated to yield the same average distance of a pollinating tree than the power exponential dispersal kernel with $\beta = 0.67$, as follows. The probability of receiving pollen from a tree located further than a distance D is assumed negligible, therefore the cumulate dispersal probability between 0 and D sums up to one, $\int_0^D 2\pi x \cdot f(x) dx = 1$ being $f(x) = a + b \cdot x$ and x the distance between trees. Then, equating the mean distance of pollen dispersal to that from the exponential function (δ), $\int_0^D 2\pi x^2 \cdot f(x) dx = \delta$, the following system of equations is obtained as a result of solving both integrals:

$$\begin{cases} \pi a D^2 - \frac{2\pi b}{3} D^3 = 1 \\ \frac{2\pi a}{3} D^3 - \frac{\pi b}{3} D^4 = \delta \end{cases}$$

which allow us the determination of the particular values for the slope and the intercept of the linear function. For $M = 137.48$ (see above) and a value of D equal to 400 m, the parameters of the linear function are $a = 5.8 \times 10^{-6}$ and $b = -1.4 \times 10^{-8}$. No greater value of D was used because the longest distance between trees in the maritime pine orchard was ~ 300 m.

Coancestry matrix

With respect to pairwise genetic relationships between trees, we first considered the simple case in which only

information about ramets identity is available. This case was implemented using a coancestry matrix with halves for ramets of the same genet and zero for the rest of the comparisons. This scenario is what previous allocation methods assumed (e.g. Bell and Fletcher 1978; Chakravarty and Bagchi 1993). Second, we estimated coancestry from molecular marker information. We obtained multilocus genotypes of 61 non-selected trees (representing each one a different genet) from the Castilian Plateau, a core location of the species in central Spain, using six highly polymorphic nuclear microsatellites (average number of alleles per locus of 10.5 and expected heterozygosity of 0.78; see Lucas et al. 2008 for technical details on nuSSR scoring). Trees were sampled randomly avoiding close neighbours following standard procedures used to collect plant material for ex situ conservation.

Three different coancestry estimators were applied: (i) molecular coancestry, defined as the probability of two alleles sampled from two different individuals being equal (*identity by state*, IBS); (ii) J. Nason’s kinship estimator (Loiselle et al. 1995), which calculated the coancestry between a pair of individuals as

$$F_{ij} = \frac{\sum_l \sum_a (p_{lai} - \bar{p}_{la})(p_{laj} - \bar{p}_{la})}{\sum_l \sum_a \bar{p}_{la}(1 - \bar{p}_{la})} + \frac{1}{2(n - 1)},$$

where p_{lai} and p_{laj} are the frequencies of the a th allele (0, 0.5 or 1) at the l th locus in the i th and j th individuals, respectively, \bar{p}_{la} the mean frequency of the a th allele in the sampled population, and n the sample size; and (iii) the method of Fernández and Toro (2006). The Nason’s approach belongs to the pairwise methods of coancestry estimation that infer the *identity by descent* (IBD) between individuals by correcting the IBS calculated from molecular markers considering the population frequencies of each allele. Alternatively, Fernández and Toro (2006) method involves the reconstruction of a feasible genealogy, yielding the pedigree-based coancestry matrix with the highest correlation with the molecular marker coancestry matrix. For this estimator, we considered coancestry matrices obtained from a single generation pedigree (FT_1, mimicking a population with low levels of relationship; average coancestry of 3.49×10^{-2}) and coancestries estimated when allowing for five-generation genealogies (FT_5, more related trees; average coancestry of 11.08×10^{-2}). Finally, a scenario where mating between ramets of the same clone is further penalised (by fixing to one the coancestry of such pairs, instead of using individual self-coancestry coefficients) was considered for the case of FT_1.

Performance testing

The global probability of generating inbred offspring was used to evaluate the performance of our method under different scenarios (simulated or real) related to this case study on maritime pine and also against traditional methods of allocating trees in ex situ conservation plantations and seed orchards. This parameter was calculated using formula (1) (under the assumption that the real coancestry matrix is the one estimated by Fernández and Toro’s method, either with one or five generations) and the whole range of power exponential pollen dispersal kernels considered in the study. Self-pollination rate was assumed to be zero, as is usual in temperate wind-pollinated trees including maritime pine (see González-Martínez et al. 2006; Lucas et al. 2008).

The high number of simulated scenarios considered and the full exploration of a case study allowed us to demonstrate the utility of our method to reduce inbreeding through tree allocation, whatever the availability of information on genetic relationships between trees (even when only clone identity was known) and irrespective of the pollination function assumed. It also permitted to evaluate the utility of genetic markers to optimise tree allocation for minimum inbreeding using molecular-based estimations of pairwise coancestry.

Results

Both simple simulated scenarios and the more complex case study showed a better performance of the novel methodology in terms of expected inbred offspring in next generation seed crop. Nonetheless, traditional methods such as *permuted neighbourhood* performed relatively well (and similarly to the newly developed method) in some scenarios (for instance, under highly leptokurtic pollen dispersal kernels). A more detailed evaluation of the methods follows.

Simulated data

Scenario 1

Table 1 shows the lowest probability of generating inbred offspring for the considered data under the *permuted neighbourhood* strategy (corresponding to accounting for three ‘rings’ or ‘neighbourhoods’ around each tree) and results under the new methodology (FG thereafter) for different sets of parameter. The assumed dispersal kernel, for the implementation of FG method as well as for the

Table 1 Probability of generating inbred offspring ($\times 10^2$), in simulated scenario 1 for a regular design, under the traditional *permuted neighbourhood* design and under the novel strategy with different values for the parameter of the power exponential dispersal kernel

	Used β	True β			
		0.2	0.67	1	2
Regular		2.91	2.90	2.58	2.18
Neighbourhood		2.90	2.88	2.52	2.07
FG method	0.2	2.89	2.87	2.46	1.98
	0.67	2.89	2.87	2.46	1.98
	1	2.89	2.87	2.46	1.97
	2	2.90	2.87	2.47	1.97

Calculations performed assuming that the real dispersal function is power-exponential with alternative β parameters and a coancestry matrix with 0.5 for comparisons between ramets of the same genet and zero for the rest of relationships

evaluation of results, was power exponential with the true values in columns and the used parameters in rows.

For all combinations of parameters, the FG design yielded better results than the neighbourhood strategy, although differences between methods were small. Surprisingly, the most intuitive plantation design consisting in repeating blocks of 4×4 individuals containing one tree of each genet led to higher probabilities of inbreeding (see values in Table 1 under the label of ‘Regular’ design).

To test the repeatability of the results for the FG method, different runs were performed beginning the simulated annealing algorithm in different starting solutions. Differences in the probability of generating inbred offspring for the corresponding plantation designs were of order 10^{-5} , and always lower than under the neighbourhood method, confirming the robustness of the optimisation methodology.

Scenario 2

For this scenario, Fig. 1 shows the corresponding plantation designs obtained by *permuted neighbourhood* methodology (only the first ‘ring’ around the tree considered) and by the novel methodology.

As the classical strategy only accounts for the relationships within clones, no ramets from the same genet were found together when applying such a method. However, up to seven times ramets from clone 1 can be found nearby a ramet from clone 2, despite clones 1 and 2 were assumed to be full-sibs. Contrarily, our method also considered the genetic relationships between genets and, therefore, avoided as much as possible to allocate ramets from clones 1 and 2 together (it only happened once). Due to the small size of the plantation site, increasing the number of ‘rings’ accounted for in the *permuted neighbourhood* method led to weird

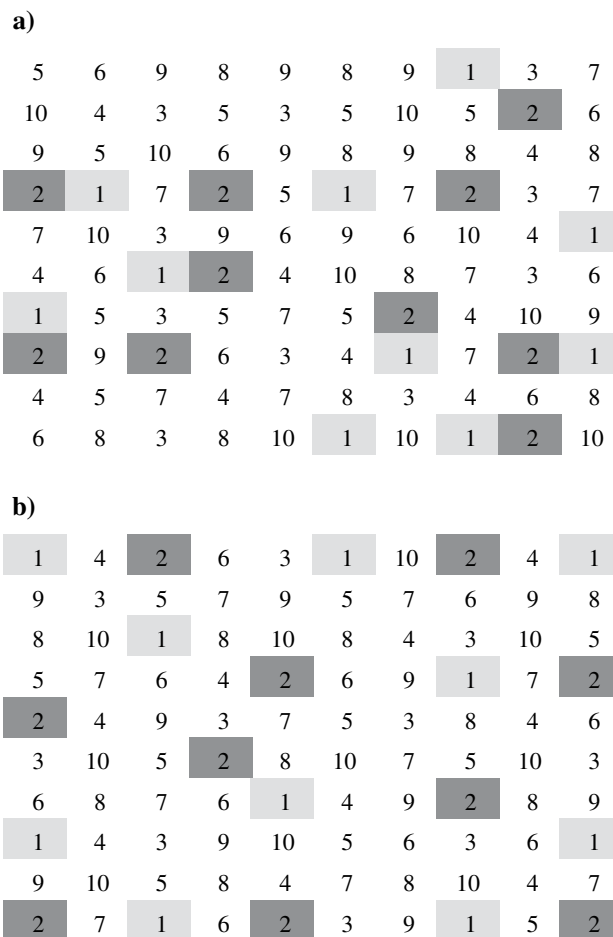


Fig. 1 Plantation design under the *permuted neighbourhood* method (a) or the novel method (b) for simulated data of Scenario 2. Clones 1 and 2 are assumed to be full-sibs

results because moving a tree far away from one of its clonal replicates put it close to another (data not shown). However, the novel method separated ramets only as long as this decreased the expected levels of inbreeding in the offspring, giving more reasonable final configurations.

Case study

The relative nature of the Nason’s kinship estimator (it provides pairwise kinship relative to the average found in the population) made it to yield negative values of coancestry within some genets and also between several genet pairs. Consequently, when the resulting coancestry matrix was used under the FG method the optimal design was planting together all ramets of those genets, as this led to low global values of the objective function (data not shown). This bizarre result would be obtained with most of the pairwise estimators. A possible solution could be transform the continuous estimates yielded by this kind of

methods to discrete genealogical relationships with the extra drawbacks it implies (see, for example, Rodriguez-Ramilo et al. 2007 for deeper insights on the subject).

The ability of the FG method to control the generation of inbreeding in open-pollinated tree plantations can be seen in Table 2, where its performance is evaluated under different sets of real parameters (in columns) when different inputs (as, in practice, these parameters are often unknown) about the relationships between individuals and about the pollen dispersal kernel were assumed (in rows). Values show the percentage of decrease in probability of generating inbred offspring relative to the value obtained under the traditional *permuted neighbourhood* design. For some scenarios, the FG method was run several times with different starting points to check the robustness of the optimisation method. Differences in the probability of generating inbred offspring were always lower than 10^{-5} , confirming the repeatability of the results.

The FG algorithm proved to be efficient in the integration of the available information, as for any particular combination of real coancestry and dispersal function best results (i.e. lower probabilities of generating inbred offspring) were obtained when true parameters were used as input values (figures in bold in each column of Table 2).

Advantage of the FG method with respect to *permuted neighbourhood* ranged from 77.89% for $\beta = 2$ and lower levels of global coancestry (case FT_1) and 0.20% for $\beta = 0.2$ and higher estimated pairwise coancestry (see Table 2). The smaller the value of the β parameter the smaller the difference with the *permuted neighbourhood* design, as fatter tails rest importance to the position of the tree within the plantation because the probability of mating is less dependent on distance between trees.

Results were better when the true coancestry matrix was obtained from FT_1 than from FT_5. In the latter case all clones were more related with no large differences among degrees of relatedness and, therefore, taking away a closely related tree was at the cost of planting another with high coancestry too. Thus, there was a lower margin for the new method to improve the plantation design.

Improvements over the classical method when the FG strategy was implemented based on the raw molecular coancestry matrix but on the correct dispersal kernel, were, obviously, smaller although trends were very similar. This is not surprising as coancestry estimates based on the reconstruction of the pedigree (FT_1 and FT_5), used in the evaluation as the true pairwise coancestry, had a high correlation with the molecular coancestry.

Table 2 Probability of generating inbred offspring ($\times 10^2$) under the traditional *permuted neighbourhood* design, and decrease in that probability, in percentage, under the novel strategy

Used parameters		True parameters							
		FT_1				FT_5			
		2	1	0.67	0.2	2	1	0.67	0.2
Neighbourhood		2.79	2.95	3.70	3.76	10.39	10.49	11.17	11.23
<i>FG method</i>									
$\beta = 2$	Clones	-0.19	2.94	1.40	0.09	-0.67	0.42	0.38	-0.02
	Molecular	40.34	10.79	-0.45	-0.19	12.59	3.78	-0.14	-0.10
	FT_1	77.89	31.53	0.53	-0.05	7.46	2.73	-0.02	-0.08
	FT_5	54.60	8.85	-0.69	-0.22	19.89	4.87	-0.16	-0.10
$\beta = 1$	Clones	-1.85	2.35	1.30	0.07	-0.19	0.66	0.36	-0.03
	Molecular	41.46	11.05	-0.38	-0.17	12.67	3.75	-0.15	-0.10
	FT_1	70.97	33.23	1.31	0.07	6.66	3.39	0.24	-0.04
	FT_5	41.95	18.30	0.78	0.00	14.83	6.70	0.30	-0.03
$\beta = 0.67$	Clones	0.84	2.80	1.68	0.15	-0.27	0.40	0.46	-0.01
	Molecular	-6.90	-19.46	0.35	0.29	2.76	-1.95	0.58	0.13
	FT_1	28.29	14.56	3.07	0.52	2.95	1.83	0.91	0.11
	FT_5	-0.34	-2.63	2.51	0.48	3.89	1.97	1.11	0.17
$\beta = 0.20$	Clones	3.01	4.42	1.80	0.18	0.07	0.74	0.50	0.00
	Molecular	-19.71	-34.52	-2.49	0.04	-0.49	-5.61	-0.04	0.10
	FT_1	19.30	5.09	2.58	0.60	1.68	0.47	0.89	0.15
	FT_5	-14.01	-15.97	1.13	0.45	1.86	-0.51	0.89	0.20

Calculations performed assuming that the real dispersal function is power-exponential with alternative β parameters and a coancestry matrix between trees obtained by the Fernández and Toro (2006) method with one or five generations (FT_1 and FT_5, respectively). See the text for further details

Notwithstanding, the novel method showed a remarkable robustness against departures of the used parameters from the true values (either the dispersal function or the coancestry matrix). Even using wrong parameters, the FG algorithm yielded better results than *permuted neighbourhood* in most cases (see Table 2).

Performance was somehow different when the only information available about relationships was the clone each tree belonged to, as can be seen in Fig. 2. In this scenario, leptokurtic pollination kernels led, as explained before, to the loss of importance of design. But differences between methods also vanished for high values of β . This behaviour is not surprising because, in this situation, only trees planted very near have a significant probability of pollinating the target tree, being the relevant distances within the first neighbourhoods and, consequently, accounted for in the classical method.

When only ramet identity is available, advantage of the novel method is clear for intermediate values of the shape parameter, as there is a higher probability of pollination for trees located not very far but outside the neighbourhoods that would be considered by other methods. Again, performance of the FG method was better with FT_1 as the true relationship matrix because there were greater differences in tree pairwise coancestry.

Logically, increasing the number of neighbourhoods used in the *permuted neighbourhood* method resulted in closer performance to the novel methodology. The influence of changing (increasing) the number of

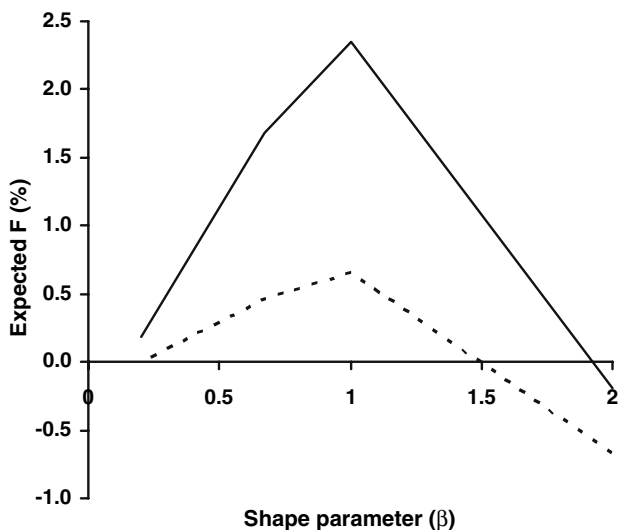


Fig. 2 Percentage of increase in expected inbreeding of the *permuted neighbourhood* method for different values of the shape parameter of the dispersal function relative to the novel method, when only ramets identity was accounted for. Solid line represents the case where real coancestry matrix was FT_1 and broken line when it was FT_5

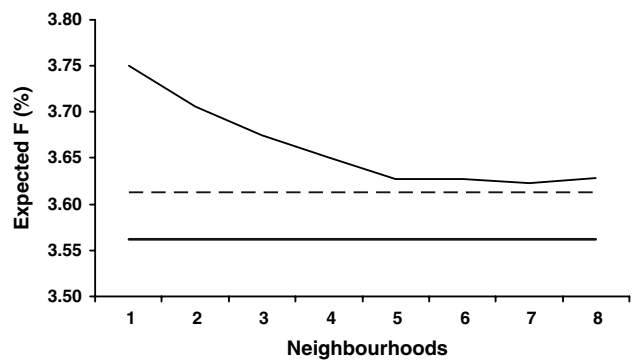


Fig. 3 Probability of generating inbred offspring (in %) under the *permuted neighbourhood* design with different number of neighbourhoods considered (thin line) and the novel method with the full coancestry matrix (thick line) or only ramets identity known (broken line). FT_1 coancestry matrix and a power exponential dispersal function with $\alpha = 24.08$ and $\beta = 0.67$ assumed

neighbourhoods where trees from the same genet are excluded can be seen in Fig. 3 for the scenario with relationships corresponding to FT_1 matrix and an exponential dispersal function with $\beta = 0.67$.

When only a few neighbourhoods were considered, probabilities of generating inbred offspring were higher than under FG method, either using a full coancestry matrix (thick line) or only clone relationships (broken line). As the number of rings increased, differences between methods relying exclusively on clone identity reduced. For this scenario, the lowest value was obtained when five neighbourhoods were accounted for, and no improvement was observed by increasing this number further.

When a linear dispersal function was assumed instead of the true exponential one, results obtained were up to 170% worse than in the optimum allocation depending on the relationship information used (clones, molecular, FT_1 or FT_5) and the true parameters for coancestry and the dispersal kernel (data not shown), yielding in general even poorer designs than the traditional strategy. This is another demonstration of the importance of determining the correct dispersal function operating in the particular species we deal with.

Figure 4 shows the plantation design proposed by the new method when the coancestry matrix was estimated by the Fernández and Toro (2006) method with a single generation (FT_1) and a power exponential pollen dispersal function with $\beta = 0.67$ was assumed. It can be observed that, in general, the method is efficient in spreading the different members of the same genet, as the classical method did (data not shown).

However, it is interesting to notice that, in some cases, self-coancestry (related to the individual inbreeding coefficient) was lower than pairwise coancestry between

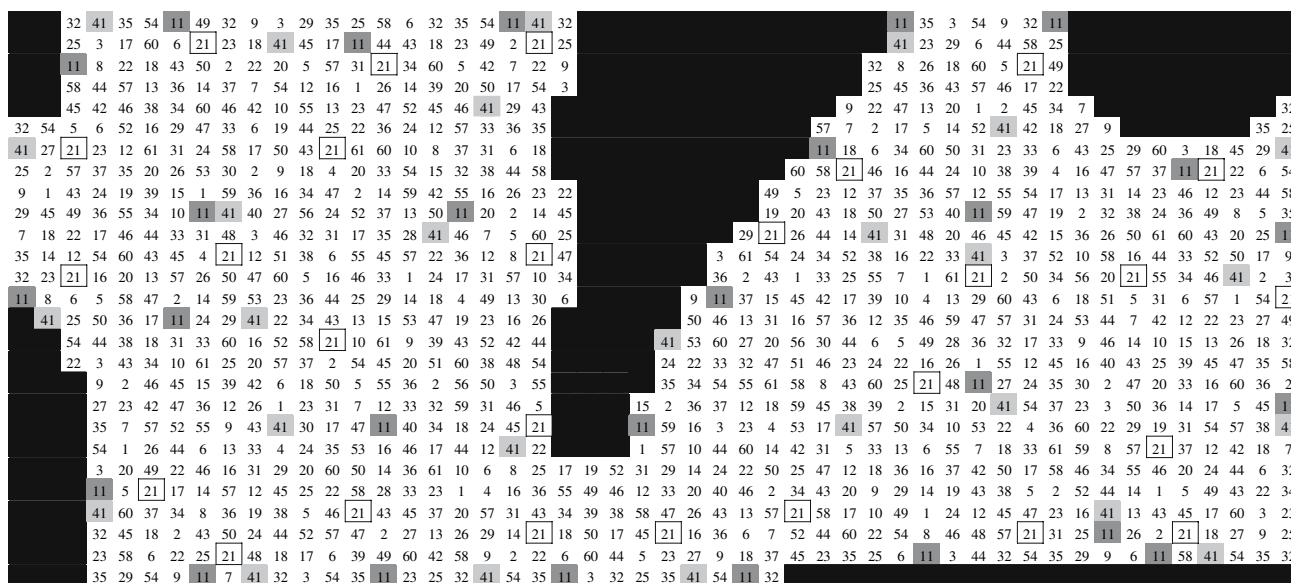


Fig. 4 Maritime pine plantation design under the novel method. Power-exponential pollen dispersal kernel with parameters $\alpha = 24.08$ and $\beta = 0.67$ and a coancestry matrix estimated from molecular

markers following Fernández and Toro (2006) approach with one generation pedigree (FT_1). More represented clones are shaded to facilitate interpretation

different genets, a feature that might be relevant in highly heterozygous organisms such as forest trees. The consequence of this fact is that the novel algorithm may allocate two individuals of the same genet near each other (see, for example, the right bottom corner of Fig. 4 where two trees from clone 11 were placed together). When coancestry matrices are estimated using a low number of markers (and, therefore, estimates of IBD from IBS may be highly biased) this situation should be avoided, for example by fixing to 1 the coancestry of ramets of the same genet, resulting in an extra penalisation of allocating two ramets of the same genet near each other. Results obtained using this ‘loaded’ matrix are shown in Fig. 5. In this scenario, the situation pointed out before did not occurred and the increase in probability of generating inbred offspring was negligible (less than 1%).

Discussion

Many temperate trees (e.g. conifers) suffer severe inbreeding depression for productive and fitness related traits but typically lack prezygotic self-incompatibility systems. This fact is particularly relevant in small isolated populations such as those created for ex situ conservation programmes. Then, avoiding inbreeding is crucial for the maintenance of ex situ tree plantations, whatever if meant for conservation purposes or developed within a tree-breeding programme.

A direct full control of the crossing design is an expensive and complicate management strategy that would not be feasible in most of the tree populations. Due to the inverse

relationship between distance and probability of pollination, a cost-effective strategy is keeping away relatives while allowing for open pollination. Classical methods, including the popular *permuted neighbourhood* implementation of Bell and Fletcher (1978), follow this principle by avoiding the allocation of trees from the same genet in the surroundings of any particular individual. However, accounting just for the close neighbourhood seems to be not enough. Different realistic pollen dispersal functions may pose more importance to trees located further. Evidence is accumulating about fat-tailed dispersal kernels (resulting in large average dispersal distances) governing pollen dispersal in both wind- and animal-pollinated forest trees (Robledo-Arnuncio and Gil 2005; Oddou-Muratorio et al. 2005; Lucas et al. 2008; see review in Smouse and Sork 2004). Moreover, these methods deny any other information about the relationship between trees but belonging to the same genet. In addition, computer implementations of traditional methods are not user-friendly, which results in many cases in sub-optimal designs.

We have developed a new method based on the minimisation of the global probability of generating inbred offspring for the whole population. Improvements of this method from the classical ones are: (i) it takes into account all the trees (whether near or far) and not only the neighbours; (ii) different pollen dispersal functions can be implemented, fitting the particularities of each species and population; (iii) it allows for the integration of all available information about the genetic relationship between trees; and (iv) it is flexible allowing for particular crosses to be banned or encouraged.

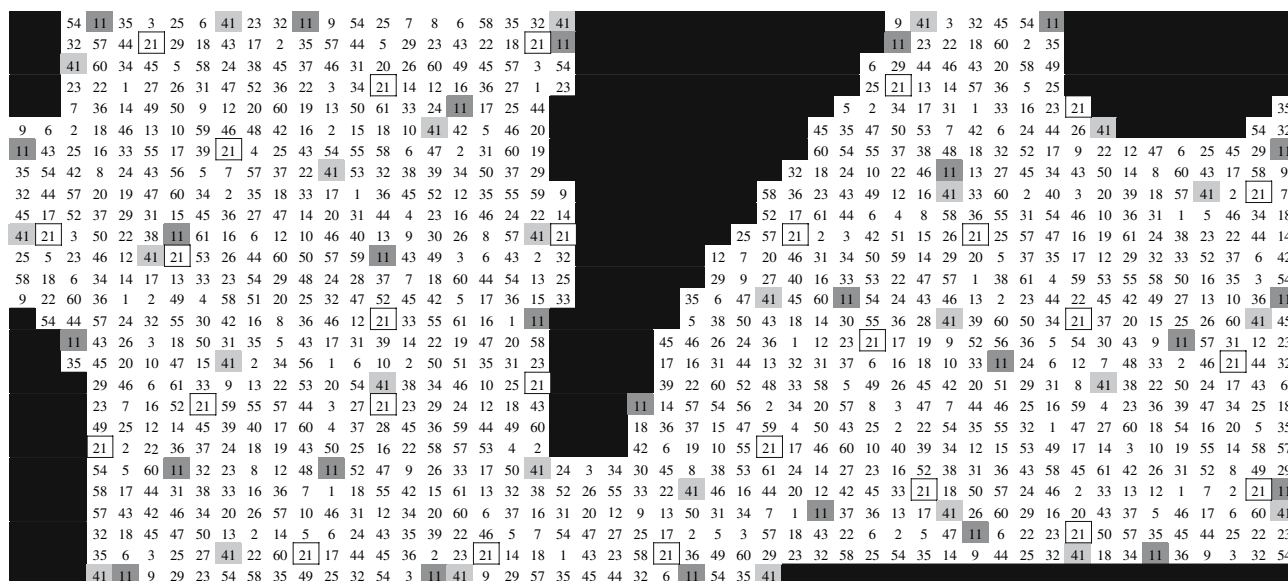


Fig. 5 Plantation design obtained using a coancestry matrix with ones for relationships between ramets of the same genet (i.e. banning the location of two ramets of the same genet near each other, irrespectively of their level of self-coancestry). The rest of parameters are as in Figure 4

Judging by our evaluation of the novel methodology, a most relevant feature seems to be the possibility of accounting for different dispersal functions, as it has been shown that different parameters led to very different solutions. Extreme values of the shape parameter (β) in power exponential kernels (i.e. highly leptokurtic distributions, $\beta \ll 1$) make irrelevant the position of the trees within the plantation, so the probability of inbreeding cannot be substantially decreased by tree allocation alone. But greater differences are to be found with highest β values because, being the closest trees the only relevant, relationships between genets become very important. Then, ex situ plantation designs for tree species with more restricted pollen flow would achieve higher benefits from the use of the optimisation strategy developed here.

The influence of the relationship information available is important too, but always mediated by the dispersal kernel assumed. In general, larger improvements will be obtained by the implementation of the new method when differential relationships exist between genets (i.e. some genets are more related to each other than with the rest of genets). This situation is likely to occur in the establishment of ex situ plantations with trees from structured/subdivided populations or based on families.

Because coancestry within genets is, in general, higher than between clones, the new method separate individuals from the same genet (as *permuted neighbourhood* methods do) but yielding lower global probability of inbreeding in the next generation, as it also accounts for the rest of relationships. Anyway, the flexibility of the method allows for the use of ‘mixed’ information, as it was shown in the case where the input for the algorithm was a matrix

with ones for relationships within clones and the known (estimated) pairwise relationships between individuals from different genets. This flexibility provides the managers with a simple and efficient tool that can be adapted to particular situations. For instance, if information does exist about undesirable crosses, as those that would result in outbreeding depression due to genotypes adapted to different local conditions (as may be the case, for example, in some marginal populations of Canary Island pine that grow in very dry environments; Vaxevanidou et al. 2006), they can be easily banned by simply modifying the input matrix by making one the kinship of banned pairs. Another case of direct application to forest trees would be the inclusion in the plantation design of information about alleles at the self-incompatibility (SI) locus (S-alleles), also easy to implement through modifying the kinship matrix. For instance, in cases where S-alleles can be scored using molecular techniques (such as for wild cherry tree; see Stoeckel et al. 2006 and references therein), relative position of trees with crosses that would be naturally incompatible can be relaxed by making zero their matrix value, irrespectively of their actual kinship.

The ability of the method to incorporate any kind of information about the genetic relationships between trees is expected to be very important for the management of conservation populations, especially in non-equilibrated situations where individuals are differentially related (i.e. some trees are much more related than the average). Notice also that the level of inbreeding in the maritime pine case study population was quite low (only ~10% of the pairwise genetic relationships estimated with molecular markers were higher than 0.125, the kinship of half-sibs)

and even better performance is expected in more inbred conservation populations. Finally, the new method is amenable to modifications accounting for other factors affecting the probability of mating, such as differences in male fecundity (for instance, due to quantity and/or quality of pollen; Burczyk et al. 1996; Oddou-Muratorio et al. 2005) or phenological overlapping (Robledo-Arnuncio et al. 2004 and references therein). A simple way to account for these factors would be to introduce weights in the probability of mating, $p(x, y)$, in equation (1) of the form: $\lambda_i \varphi_{ij} / \sum_{k \in N} \lambda_k \varphi_{kj}$, where λ_i is the male fecundity of the i th individual, φ_{ij} the phenological overlapping of the i th pollen donor with the j th mother tree and N is the total number of trees in the plantation.

The conclusion from the present study is that the new method can be a powerful tool for plantation design in the tree breeding and ex situ conservation context, replacing with advantage current traditional approaches. It can also facilitate the integration of ecological, life history and pedigree information in ex situ conservation strategies, and encourage managers to adopt objective-oriented plantation designs. A user-friendly computer program to perform the calculations will be made available upon request.

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