

Preserving Population Allele Frequencies in Ex Situ Conservation Programs

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Abstract: Optimization of contributions of parents to progeny by minimizing the average coancestry of the progeny is an effective strategy for maintaining genetic diversity in ex situ conservation programs, but its application on the basis of molecular markers has the negative collateral effect of homogenizing the allelic frequencies at each locus. Because one of the objectives of a conservation program is to preserve the genetic composition of the original endangered population, we devised a method in which markers are used to maintain the allele frequency distribution at each locus as closely as possible to that of the native population. Contributions of parents were obtained so as to minimize changes in allele frequency for a set of molecular markers in a population of reduced size. We used computer simulations, under a range of scenarios, to assess the effectiveness of the method in comparison with methods in which contributions of minimum coancestry are sought, either making use of molecular markers or genealogical information. Our simulations indicated that the proposed method effectively maintained the original distribution of allele frequencies, particularly under strong linkage, and maintained acceptable levels of genetic diversity in the population. Nevertheless, contributions of minimum coancestry determined from pedigree information but ignoring the genealogy previous to the conservation program, was the most effective method for maintaining allelic frequencies in realistic situations.

Keywords: allelic diversity, artificial selection, gene diversity, genetic drift, inbreeding, molecular markers, quantitative variation

Preservación de las Frecuencias Alélicas en Programas de Conservación Ex Situ

Resumen: La optimización de la contribución de padres a la progenie mediante la minimización de del parentesco promedio de la progenie es una estrategia efectiva para el mantenimiento de la diversidad genética en programas de conservación ex situ, pero su aplicación utilizando marcadores genéticos tiene el efecto colateral negativo de producir la homogeneización de las frecuencias alélicas en cada locus. Puesto que uno de los objetivos de un programa de conservación es la preservación de la composición genética original de la población amenazada, diseñamos un método en el que se utilizan marcadores para mantener la distribución de la frecuencia alélica en cada locus lo más cercanamente posible a la de la población nativa. Se obtuvieron las contribuciones de los padres que minimizan los cambios en la frecuencia alélica para un conjunto de marcadores moleculares en una población de tamaño reducido. Utilizamos simulaciones por ordenador, cubriendo diversos escenarios, para evaluar la efectividad del método en comparación con métodos con los que se buscan contribuciones de mínimo parentesco, ya sea usando marcadores moleculares o información genealógica. Nuestras simulaciones indicaron que el método propuesto mantenía de forma efectiva la distribución original de las frecuencias alélicas, y mantenía niveles aceptables de diversidad genética en la población. Sin embargo, las contribuciones de mínimo parentesco, determinadas a partir de

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información del pedigrí pero ignorando la genealogía previa al programa de conservación, fue el método más efectivo para el mantenimiento de las frecuencias alélicas en situaciones realistas.

Palabras Clave: deriva génica, diversidad alélica, diversidad génica, endogamia, marcadores genéticos, selección artificial, variación cuantitativa

Introduction

Preservation of genetic diversity and avoidance of inbreeding are primary issues in conservation programs for threatened and endangered species. A loss of genetic diversity and an increase in inbreeding may result in inbreeding depression, lack of adaptation to changing environmental conditions, and population extinction (Hedrick 2001; Frankham et al. 2002). There is an increasingly general consensus on the best way to preserve genetic diversity and to avoid inbreeding in ex situ conservation programs. The most widely accepted strategy minimizes the global coancestry (kinship) in the population through optimization of contributions of parents to the next generation (e.g., Ballou & Lacy 1995; Lacy 1995, 2000; Montgomery et al. 1997; Caballero & Toro 2000). Under a particular hierarchical structure and simplified conditions, the method reduces to equalizing family sizes (Gowe et al. 1959; Wang 1997; Sánchez-Rodríguez et al. 2003). This procedure of finding contributions of minimum coancestry (MC method hereafter) maximizes the population genetic diversity in terms of expected heterozygosity and effective population size (Fernández & Toro 1999; Caballero & Toro 2000, 2002) and is flexible and robust against departures from the ideal conditions (Fernández et al. 2003). Nonrandom mating systems can be applied subsequently to selected animals resulting from the optimization (Caballero et al. 1996; Sonesson & Meuwissen 2000; Meuwissen 2007), but the impact of the mating system on the maximization of the population genetic diversity is generally much lower than that of the parents' contribution.

The MC method was originally proposed for application to genealogical information. Nevertheless, pedigree records are often unavailable or difficult to obtain, as for species with complex recording procedures (e.g., in aquaculture programs or colony breeding species). In these situations, molecular marker data can then be used as an alternative. Previous theoretical studies show that the method applied with marker data maintains its effectiveness in conserving global gene diversity (expected heterozygosity) and that it effectively maintains allelic richness in a population (Fernández et al. 2004). Nevertheless, because contributions of minimum coancestry lead to the highest expected heterozygosity, and this latter is the highest under equal allele frequencies, the method tends to homogenize allelic frequencies within loci. If rare alleles are led toward intermediate frequency values, their probability of loss is reduced, which ex-

plains why the method is effective in avoiding allele losses (Fernández et al. 2004).

In the context of an ex situ conservation program, this may have a negative consequence. It can be argued that a conservation program should preserve genetic variation as closely as possible to that of the original population (Lacy 2000), particularly if reintroduction to the wild is an expected outcome. Adaptation to captivity is an undesirable consequence of conservation programs, which may change the genetic composition of the populations (Frankham 2008). In addition, if deleterious mutations maintained at low frequencies in natural populations increase in frequency because of the conservation method, the reproductive performance of the population after reintroduction may be affected (Schoen et al. 1998; Fernández & Caballero 2001; Theodorou & Couvet 2003; Rodríguez-Ramilo et al. 2006). For domestic plants or animal species, it may also be desirable that certain characteristics previously selected for be maintained under relaxed selection during a period of conservation.

We devised a conservation strategy in which information from molecular markers is used specifically to maintain the allelic frequencies of the native original population. We used computer simulations to evaluate performance of the method in terms of maintenance of allelic frequencies and maximization of gene and allelic diversity in comparison with the MC method applied either with genealogical or marker data.

Methods

Maintaining Original Allelic Frequencies

Our objective was to maintain as closely as possible the genomic allelic frequencies present in the original native population. Thus, we needed to define a measure of "distance" between the actual (observed) frequencies and the original (desired or expected) ones. We used the Kullback-Leibler (KL henceforth) divergence criterion, which measures the goodness of fit or the relative entropy between 2 discrete probability distributions (Kullback 1997). We assumed that N individuals of a given population are genotyped for a set of M molecular marker loci with K_m alleles at a given marker m . The KL divergence between the current allele frequencies and the original ones was estimated as

$$KL = \sum_m^M \sum_k^{K_m} P'_{km} \log \left(\frac{P'_{km}}{P_{km}} \right), \quad (1)$$

where p_{km} is the frequency of allele k of locus m in the original population, and p'_{km} is the corresponding frequency expected to occur in the next generation following a particular scheme of contributions (i.e., a particular number of offspring per available parent). The latter can be calculated with the expression

$$p'_{km} = \left(\sum_{i=1}^N c_i g_{mki} \right), \quad (2)$$

where N is the number of individuals, c_i is the relative contribution of individual i to the next generation, and g_{mki} is the proportion of successful gametes carrying allele k of marker m transmitted by this individual (1 for homozygotes, 0.5 for heterozygotes, and 0 for noncarriers). To get absolute contributions, relative ones were multiplied by $2N$. Therefore, the method (KL method hereafter) consisted of finding, in each generation, the optimal contributions from parents (number of offspring that each individual leaves to the next generation) that minimize Eq. 1 for a given number of neutral molecular markers genotyped in the population. As for other strategies relying on molecular marker information, the idea is that the effect that the method produces on the markers be extended, at least partially, to the whole genome.

Contributions of Minimum Coancestry

We compared the KL method with the widely recognized MC method (e.g., Caballero & Toro 2000). This strategy optimized contributions from parents by minimizing the global coancestry weighted by those contributions,

$$MC = \sum_{i=1}^N \sum_{j=1}^N c_i c_j f_{ij}, \quad (3)$$

where c_i is the relative contribution of individual i to the next generation, f_{ij} is the coancestry between individuals i and j , and N is the number of breeding individuals.

The method has been applied with pedigree coancestries and with coancestries deduced from neutral molecular markers genotyped in the population (molecular coancestry). The molecular coancestry between 2 individuals is defined as the probability that 2 genes taken from each of them for a given marker locus are identical (Malécot 1948), and these probabilities were averaged for the whole set of markers used.

Genotyping Offspring

The MC and KL methods provided the optimal contributions from parents that minimize the expected coancestry or the expected KL divergence of allele frequencies in the progeny, respectively. The methods were, therefore, applied to the potential parents before any progeny appeared. An alternative situation, however, is possible for species with a high reproductive output, in which the available parents can produce a sufficiently large number

of offspring from which individuals can be selected to be kept as breeders for the next generation. Thus, in this case the KL and MC methods can be applied to the genotyped offspring, choosing as parents those offspring that have a minimal observed coancestry or KL divergence of allele frequencies. In the case of minimum coancestry determined on the basis of pedigrees, contributions from parents or progeny data are identical because the expected relationship between the members of a family is identical for all of them. Nevertheless, results derived from markers can differ depending on whether the criteria are applied to parents or progeny. Because for the latter the methods act on observed data rather than on expectations, they are bound to perform better than when only parents are genotyped.

Simulation Procedure

We simulated a population size (N) of 64 individuals (half of each sex), kept constant over generations. A population size of 32 was also simulated, but the results were similar and are not shown. These relatively low population sizes were chosen to represent small populations of species kept in captivity. The genome of individuals consisted of 20 chromosomes (a typical lower-bound value in mammals; Van Vleck et al. 1987), each with 20 evenly spaced loci. Half of these loci were multiallelic neutral marker loci and the other half were biallelic loci assumed to control a given quantitative trait. Neutral markers and quantitative trait loci were allocated in alternating positions in the chromosome. We assumed chromosome lengths (L) were $L = 1$ and 0.1 Morgan. The first can be considered a typical value because a minimum of 1 crossover per chromosome arm is thought to occur in sexual taxa (Pardo-Manuel de Villena & Sapienza 2001). The second represents an extreme situation of small genomes or genome regions of low recombination. When obtaining gametes a Poisson distributed random number of crossovers with mean L was generated in randomly chosen places on each chromosome without interference. Genealogical relationships were also recorded for all generations.

In the initial generation individuals were assumed unrelated and not inbred. Therefore, for the neutral markers, individuals carried 2 different alleles at each locus (i.e., there were initially $2N$ different alleles per locus), which allowed us to estimate probabilities of identity by descent from marker alleles in the subsequent generations. The initial allele frequency for quantitative trait loci was binomially distributed with mean 0.5. All quantitative trait loci had equal effects with additive within and between locus gene action. The initial heritability for the quantitative trait was 0.4.

To obtain a starting population with a previous history and some degree of relatedness among individuals, the population was run for a number of generations

previous to the establishment of the conservation program. In one scenario the population was maintained for 35 unmanaged generations with random mating and no selection of parents (all individuals had the same probability of producing progeny). In another scenario 5 generations of artificial selection for the quantitative trait were carried out in the population. In each generation one-eighth of the individuals of each sex with the highest phenotypic value for the quantitative trait were selected to be the breeders for the next generation. Cases with lower selection intensity (selection of one quarter of the individuals) were also considered, but they yielded similar results and thus are not shown. In both historical scenarios the starting global molecular coancestry of the population was about the same (approximately 0.25). Optimal contributions from parents were first obtained according to each specific method, and then the progeny was obtained by random mating of parents.

After the initial 5 (unselected or selected) or 35 generations, the conservation program started (generation zero), and 5 treatments were applied for 15 discrete generations.

1. method KL_M , optimal contributions from parents, which were obtained from marker information, to minimize KL divergences (Eq. 1) between current allele frequencies and those at generation zero for neutral molecular markers;
2. method MC_M , optimal contributions from parents to minimize global molecular coancestry (Eq. 3) for neutral molecular markers;
3. method MC_P , optimal contributions from parents to minimize global pedigree coancestry (Eq. 3), with coancestries from all generations (i.e., including the previous 5 or 35 generations);
4. method MC_P^* , optimal contributions from parents to minimize global pedigree coancestry (Eq. 3), assuming the genealogical relationships previous to the start of the conservation program are unknown; and
5. control, an unmanaged control used for reference.

We based methods MC_M and KL_M on information from 1, 2, or 5 neutral markers for each chromosome (i.e., 20, 40, or 100 markers for the whole genome) uniformly distributed along the chromosome (i.e., in the case of 1 marker per chromosome, this was allocated in the middle of the chromosome; in the case of 2 markers, these were at one- and two-third distances from the tip, etc.). The remaining neutral genes (up to 200) were used for evaluation (see later).

In the scenarios where the conservation criteria were applied to the offspring rather than on the parents, we assumed that the number of progeny available for evaluation and selection was 4 (total $2N$ progeny), 8 ($4N$), or 16 ($8N$) per parent. Results with $4N$ and $8N$ evaluated offspring gave results not very different from those with $2N$ offspring, except that computing time was longer be-

cause of the larger number of progeny. Thus, only results for the latter are shown.

In most cases no artificial selection was carried out for the quantitative trait during the conservation program. Nevertheless, to simulate adaptation to captivity a situation was run in which no selection was applied previous to the start of the program and artificial selection (selection of one-half of the individuals) was applied during the conservation period. For the unmanaged control, mass selection was carried out on the quantitative trait, whereas selection within families was performed for the conservation methods.

For every generation we estimated several parameters in the population on the basis of the neutral genes not used as markers in the application of the above conservation criteria: (1) Kullback-Leibler (KL) divergence between current frequencies and initial (generation zero) frequencies, (2) average global gene diversity (GD) (i.e., the heterozygosity expected under Hardy-Weinberg equilibrium) obtained as 1 minus the average global molecular coancestry, and (3) average number of alleles per locus. For the unmanaged control reference and for minimum coancestry applied to pedigree information (MC_P and MC_P^*), all the above parameters were evaluated from the same neutral genes as for the other methods. The phenotypic mean and variance for the quantitative trait was also estimated every generation. Each case was replicated between 100 and 1000 times, depending on the computing time, and results were averaged among replicates.

All optimizations were performed with the simulated annealing algorithm (Kirkpatrick et al. 1983; Press et al. 1989) under the following restrictions: only integer non-negative solutions were allowed; the sum of all contributions (or all selected offspring) equaled $2N$ to maintain a constant population size; and half the contributions came from each of the sexes (for details, see Fernández & Toro 1999). The simulation program was written in C language and is available from the authors on request.

Results

The average genomic KL divergence between the observed allele frequencies and the original (generation zero) frequencies was independent of the degree of linkage for the unmanaged control and the MC_P (and MC_P^*) method because information on markers was not involved in their development (cf. Figs. 1a & 1c vs. Figs. 1b & 1d). The minimum coancestry method applied to markers (MC_M) produced a relatively high KL divergence from the original frequencies. This was sometimes even larger than for the unmanaged control in the early generations. The reason is that MC_M homogenized the allele frequencies within loci

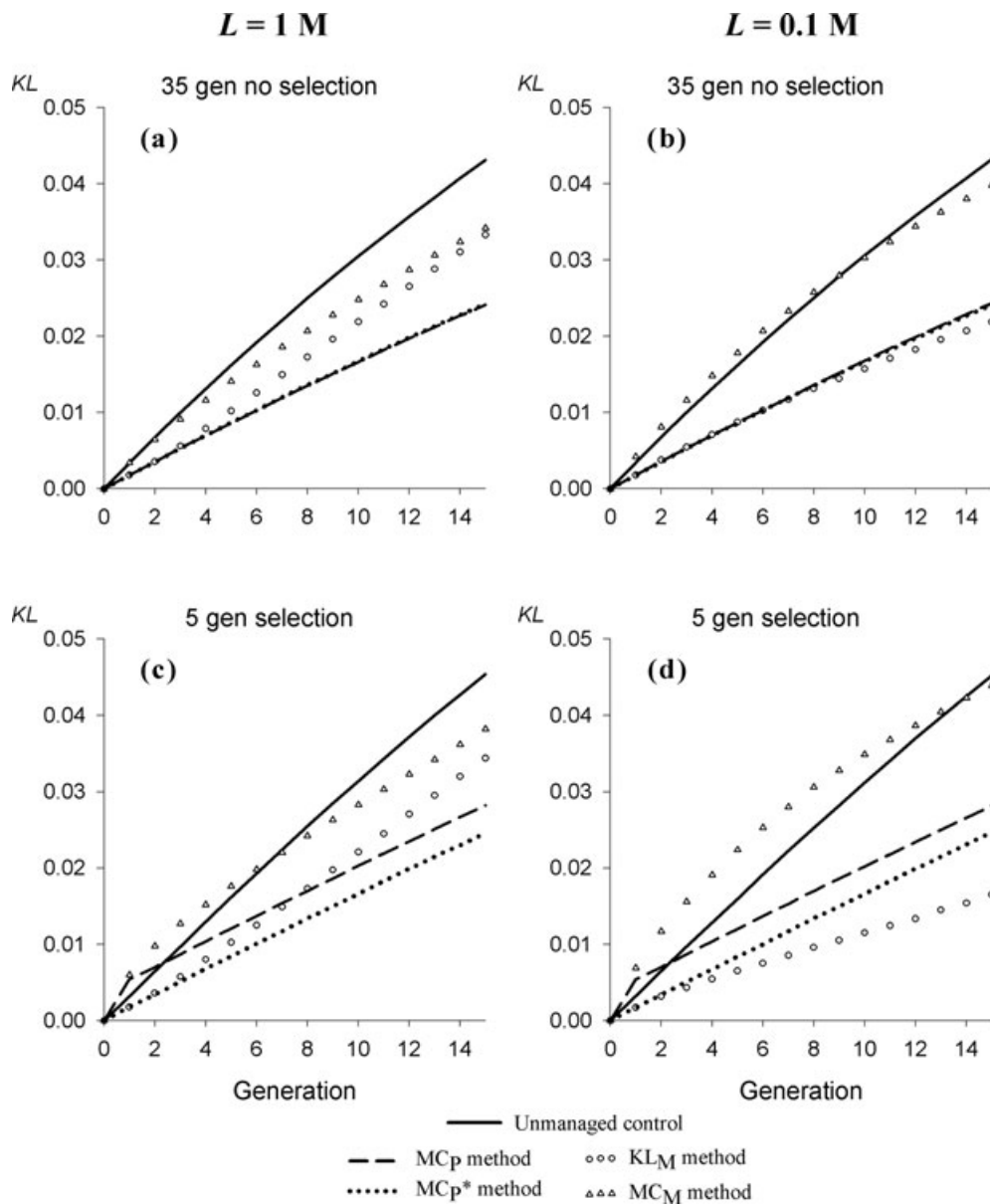


Figure 1. Average genomic Kullback-Leibler (KL) divergence between allelic frequencies of a set of neutral genes and their original (generation zero) values in a conservation program lasting 15 generations (KL_M , contributions from parents sought to minimize the KL divergence of 5 markers per chromosome on 20 chromosomes; MC_M , contributions from parents sought to minimize global molecular coancestry from the same set of markers as for the KL_M method; MC_P , minimum coancestry method applied to pedigree data considering the genealogical relationships previous to the start of the conservation program; MC_P^* , same as MC_P , but ignores the genealogical relationships previous to the start of the conservation program; control, unmanaged control population, used for reference; chromosome length in Morgans (M) of each of the 20 chromosomes). In (a) and (b), there is no selection in 35 generations previous to the start of the conservation program. In (c) and (d), 5 generations of artificial selection for a quantitative trait controlled by QTLs (quantitative trait loci) linked to the neutral genes occurred before the start of the program. Markers used for application of the methods KL_M and MC_M were not used in the estimation of the average KL divergence of the genome. Standard errors are below 0.0003.

because gene diversity for the markers reached their maximum value at intermediate frequencies (recall that the maximum expected heterozygosity at a locus occurs when the alleles are at equal frequencies).

The new method (KL_M) produced the lowest average KL divergence for tight linkage ($L = 0.1$ M). Nevertheless, minimum pedigree coancestry (MC_P) and, particularly, MC_P^* , which ignores the genealogy previous to the

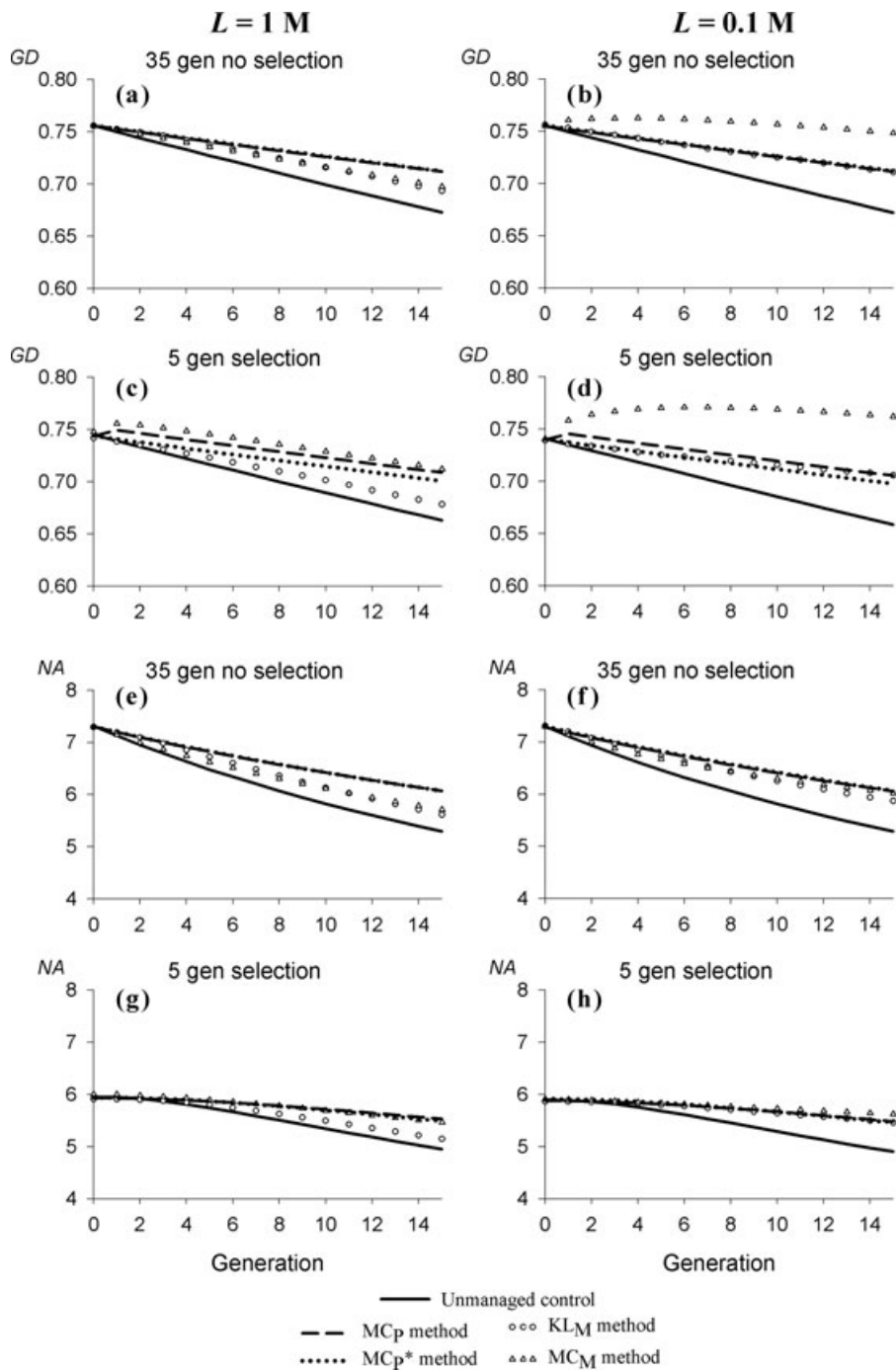


Figure 2. Average gene diversity (GD) and number of alleles segregating (NA) in the conserved population for the cases presented in Fig. 1. Markers used for the application of the methods KL_M and MC_M were not used in the estimation of GD and NA. Standard errors of GD and NA are below 0.004 and 0.022, respectively.

conservation program, were more effective for a realistic value of $L = 1 M$ (Figs. 1a & 1c).

For tight linkage ($L = 0.1 M$), minimum coancestry applied to markers (MC_M) maintained the highest overall gene diversity (Figs. 2b & 2d), clearly above that maintained by minimum coancestry applied to the pedigrees (MC_P or MC_P^*). As linkage got looser, however, differences between MC_P and MC_M were reduced (Figs. 2a & 2c) and even reversed (i.e., MC_P maintained more global gene diversity than MC_M). The KL_M method also main-

tained values of gene diversity higher than those under the unmanaged control, which suggests that the new method has some effectiveness in maintaining overall gene diversity.

The MC_P (or MC_P^*) method was the most effective alternative for maintaining allelic richness (average number of alleles per locus; Figs. 2e-h) except, perhaps, under tight linkage and previous selection (Fig. 2h). Nevertheless, with tight linkage ($L = 0.1 M$) all management methods performed very close to one another,

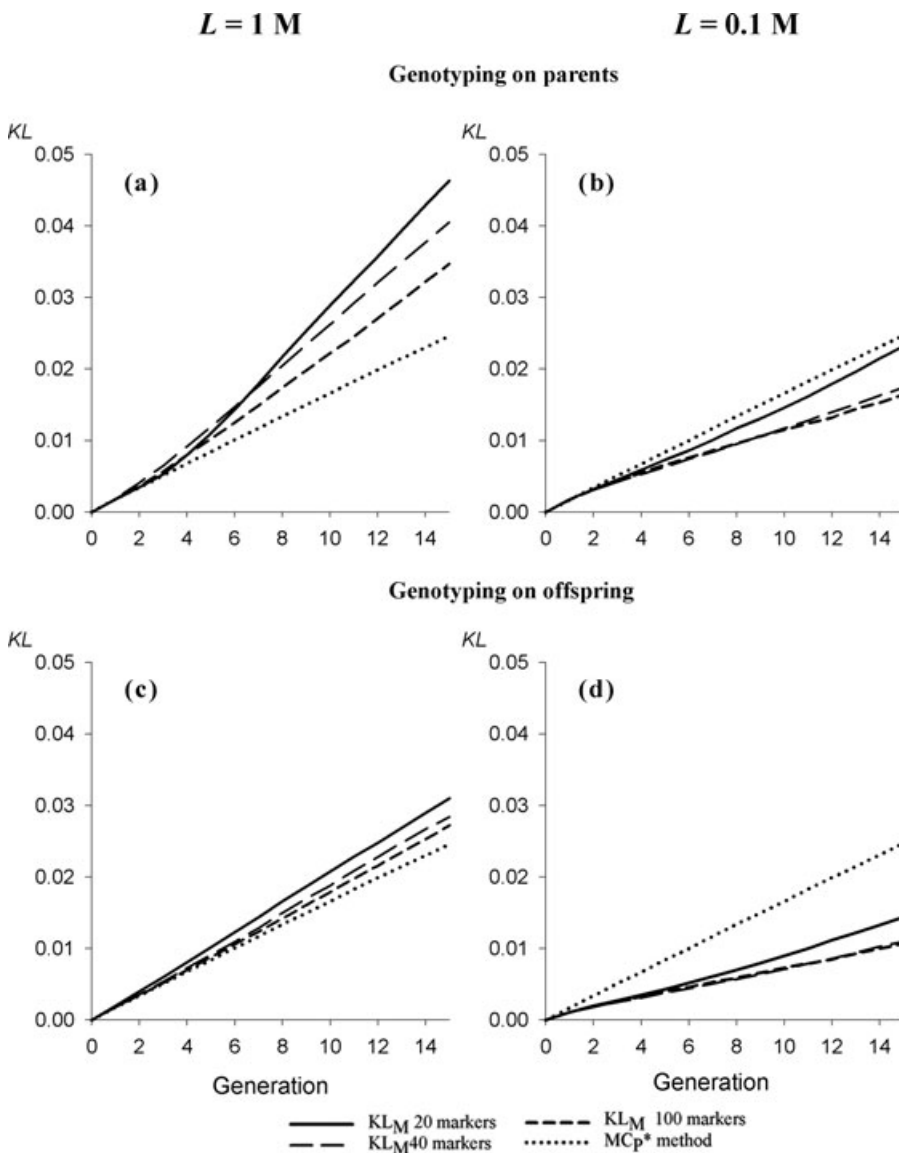


Figure 3. Average genomic Kullback-Leibler (KL) divergence between the current allelic frequencies and their original (generation zero) values under the KL_M management method (applied to 100, 40, or 20 loci per genome) for a scenario in which chromosome lengths are $L = 0.1$ or 1 Morgan and 5 generations of artificial selection have been carried out before the conservation program. The MC_P^* method (described in text) is also presented for reference. In (a) and (b), parents were genotyped so that their optimal contributions were sought. In (c) and (d), progeny was genotyped so that a set of breeders was selected from this progeny to minimize the KL divergence. Standard errors are below 0.0001.

maintaining a larger number of alleles than the unmanaged control.

The use of a smaller number of markers (1 or 2 per chromosome, that is, 20 or 40 per genome, respectively) clearly reduced the effectiveness of the KL_M method under loose linkage (Fig. 3a), but under tight linkage, the number of markers became irrelevant when about 40 markers or more were used (Fig. 3b). As expected, genotyping of offspring implied an improvement of the KL_M method with respect to the use of parents (cf. Figs. 3a & 3b and Figs. 3c & 3d). Nevertheless, with loose linkage ($L = 1$ M), MC_P^* still maintained a lower KL divergence than that from the KL_M method (Fig. 3c).

We investigated application of the different management strategies in the case where the conserved population was artificially selected previously for a quantitative trait of interest and the objective was to keep the population's quantitative-trait loci allelic frequencies as

close as possible to the original ones. The MC_M method implied a loss in the genetic gain obtained in the population, particularly under strong linkage (Figs. 4a & 4b), whereas the KL_M method and MC_P (or MC_P^*) method maintained the original mean with a standard deviation (2.06×10^{-3} and 2.18×10^{-3} , respectively) slightly lower than that for the unmanaged control (2.21×10^{-3}).

Finally, we also considered the situation in which no selection occurred previous to the conservation program, but some adaptation to captivity (selection for the quantitative trait loci) occurred during the program. All conservation methods reduced the selection response to about one-half that of the unmanaged control, because they implied only selection within families, but no appreciable difference between methods was found for loose linkage. For tight linkage ($L = 0.1$ M), however, the KL_M method showed a slightly better performance.

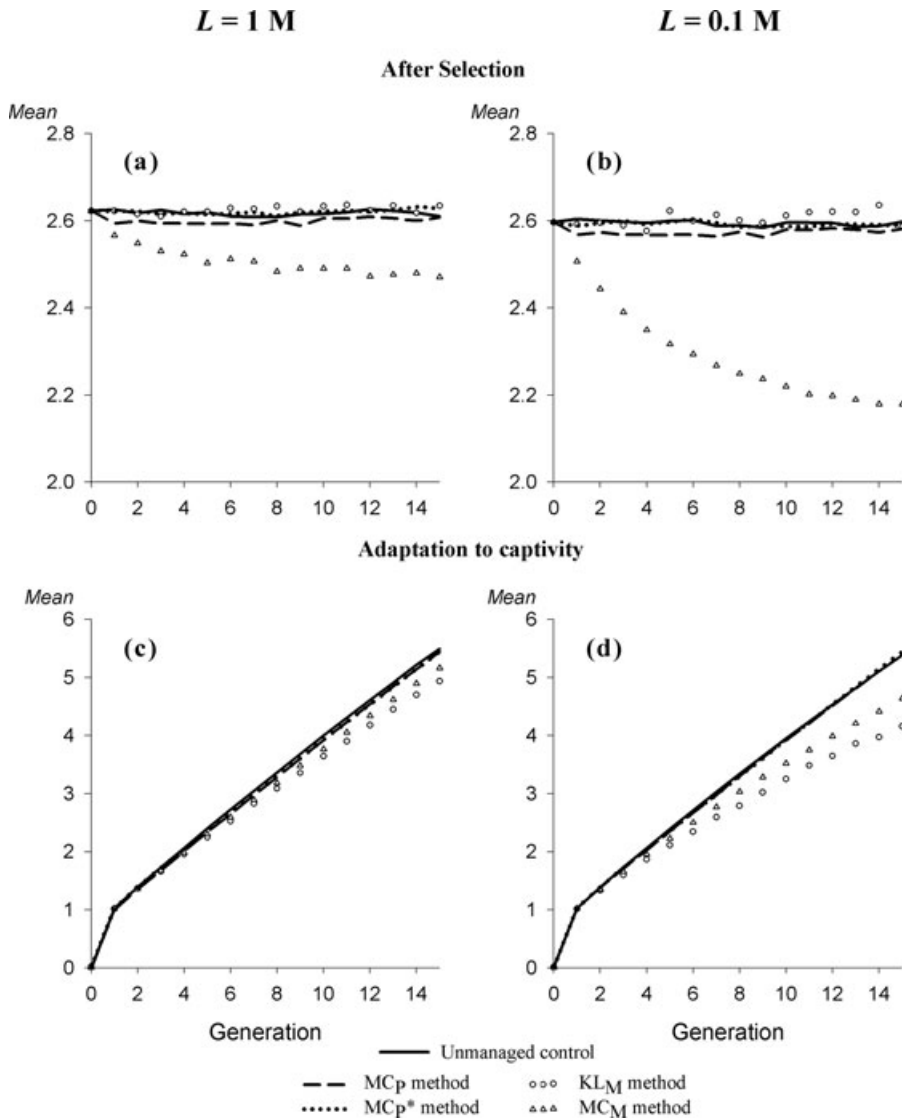


Figure 4. Mean phenotypic values for a quantitative trait after application of the different conservation criteria (see Fig. 1). In (a) and (b), the trait was selected for 5 generations before the start of the conservation program. In (c) and (d), the trait was selected during the conservation program to simulate adaptation to captivity. In both cases the objective was to maintain the distribution of allelic frequencies at the start of the program (generation zero). Standard errors are below 0.0024.

Discussion

The results of our simulations showed that maintaining minimum pedigree coancestries (MC_P method and, particularly, MC_P^{*}, which ignores the previous genealogical relationships) is probably the most comprehensively effective conservation method. This is, in fact, the method generally recommended in conservation breeding programs (e.g., Ballou & Lacy 1995; Caballero & Toro 2000). Under realistic levels of linkage ($L = 1$ M per chromosome), this method maintained the lowest KL divergences from the original allelic frequencies (Figs. 1a & 1c, Figs. 3a & 3c), the highest levels of allelic diversity (Figs. 2e & 2g), and the highest levels of gene diversity under no previous selection (Fig. 2a). This method works with pedigrees and thus produces good results because it acts on the whole genome. In contrast, the methods in which we used a (necessarily) limited number of markers (MC_M and KL_M) were not always so effective in extending their effects to the whole genome. The fact that the

minimum-pedigree-coancestry method maintained lower divergences from the original allelic frequencies than a method in which we used markers specifically developed for that (KL_M) is in parallel with a similar result found previously regarding the maintenance of the allelic richness. Fernández et al. (2004) found that the MC_P method maintains the number of alleles more effectively than a method that used markers specifically designed to maintain alleles.

Interestingly, the minimum pedigree coancestry method performed even better for the cases of 5 generations of previous selection, when the previous genealogical relationships were ignored (MC_P^{*}). This occurred because, under selection, genealogical relationships are not perfectly coupled with true genomic relationships (Fernández et al. 2000). Therefore, considering pedigree relationships can produce undesired results. The fact that ignoring previous pedigree records worked so well is a great advantage because they are usually not available.

It was only for cases of strong linkage when the MC_P and MC_P^* methods performed much worse than the KL_M method (in terms of KL divergences; Figs. 1b & 1d) and the MC_M method (in terms of gene diversity; Figs. 2b & 2d). In these situations of close linkage, application of the methods to a limited number of markers successfully affected other linked genes, extending effects of the conservation criteria to the whole genome. Nevertheless, the situation in which chromosomes length is 0.1 Morgan is more a pedagogical scenario than a realistically general one.

Minimum coancestry contributions derived from markers (MC_M) strongly distorted the original distribution of gene frequencies of the base population (Fig. 1). This occurred because minimum molecular coancestry is a form of selection and, therefore, it produces changes in allelic frequencies, leading them toward intermediate values (Fernández et al. 2004). This was not the case when minimization of coancestry was derived from pedigrees (MC_P or MC_P^*) because then selection was not involved and the method acted only to minimize genetic drift. This performance of MC_M , distorting the original distribution of allelic frequencies, argues against its use in conservation programs.

In some situations pedigrees cannot be easily recorded even in captive conditions. This is, for example, the case in aquaculture settings, where crosses are usually made between 1 female and semen of many males (e.g., Wedekind et al. 2007), which complicates recording relationships in the progeny. It is also the case in supportive breeding programs, where genealogical relationships of all individuals cannot be recorded. In these scenarios the use of markers can be essential, and the KL_M method could be considered an alternative to MC_M because it would produce little distortion in the original distribution of allelic frequencies and avoid some loss of gene and allelic diversity. We considered the Kullback-Leibler divergence the control parameter in the conservation criterion for several reasons. Simpler measures, such as mean square error, were not appropriate because allelic differences were treated equally at any original frequency. Thus, they paid no special attention to low-frequency alleles at risk of being lost. We considered chi-square divergence as an alternative (the squared difference between current and original frequencies divided by the original frequency). This parameter gave more importance to deviations from original frequencies of those alleles already at low frequency. The performance of this criterion was similar to that of the KL_M method except that the number of alleles maintained was larger for the latter (not shown).

One argument against maintaining the original distributions of allelic frequencies is that the number of founders may be too small in some scenarios for species under the risk of extinction, which would provide a poor representation of the original allelic frequency distribution.

Nevertheless, there are other situations, such as in supplementation programs, where the population sizes are not necessarily small, and maintaining the original distributions of allelic frequencies would not be such a problem. Use of a method that tries to keep the original allelic frequencies is justified if a conserved population has been previously selected for one or more quantitative traits of interest. Whereas global genomic diversity should be maintained during the conservation period, it may also be important not to lose the achieved response for the quantitative traits. This was the idea behind the simulation results presented in Figs. 4a & 4b. Clearly, minimum coancestry determined from markers (MC_M method) did not meet this requirement, and part of the genetic gain previously obtained was lost during the conservation period, whereas both MC_P (and MC_P^*) and KL_M achieved this goal. Another reason for using a method that maintains the original allelic frequencies is to avoid adaptation to captivity (see Frankham 2008). All conservation methods that implied only selection within families were effective in reducing adaptation to captivity, although the KL_M was somewhat more effective under tight linkage (Figs. 4c & 4d).

Because molecular minimum coancestry (MC_M) maintained lower levels of coancestry than the KL_M method and this produced a lower KL divergence, a question arises as to whether these 2 criteria could be combined. A joint objective function could be assumed with a term related to the measure of KL divergence and another related to coancestry of the parents. Moreover, depending on the particular interest of the managers each term could be weighted differentially, giving more importance to maintenance of the original allelic frequencies or maintenance of global gene diversity. The suitability of such combined functions has been proved, for example, in the establishment of semen banks within the framework of an eradication program (Fernández et al. 2006).

The use of molecular markers to help in maintenance of genetic diversity is an old idea. Chevalet (1992) proposed to select as parents for the next generation the individuals more heterozygous for several loci. Toro et al. (1998) showed that this strategy performs worse than a strategy based on frequency-dependent selection. In their proposal, the value attached to the genotype A_iA_j at each locus is $(1 - p_i/2)(1 - p_j/2)$, p_i and p_j being the frequencies of the A_i and A_j alleles, respectively, and the value of an individual is the sum over all marker loci. Nevertheless, there are many other possible ways of implementing frequency-dependent selection. Toro et al. (1999) suggest that the most natural form of frequency-dependent selection would be to minimize the molecular coancestry of the group of selected individuals or, in other words, to maximize its expected heterozygosity. The last refinement of this procedure is the MC_M method we considered here. Nevertheless, our results showed

that this is not a useful method from the point of view of conserving the original distribution of allelic frequencies and that either the KL_M method or minimum pedigree coancestries, whether or not pedigrees are available, should be used instead.

For realistic levels of linkage, contributions of minimum coancestry determined on the basis of pedigrees, ignoring past relationships, is the most effective method for maintaining original allelic frequencies, and it maintains high levels of gene and allelic diversity. Minimum coancestry that takes into account all genealogical information is somewhat less effective in maintaining the original allelic frequencies, but more effective in terms of maintaining gene diversity. In situations where pedigree records are available, markers may perhaps be used to deduce missing genealogical relationships of individuals. Nevertheless, in situations where pedigree records are difficult to obtain, such as in supportive breeding programs, and for some scenarios (e.g., aquaculture settings, colony-breeding species), molecular markers should be used, and the proposed KL_M method would be a better alternative than minimum coancestry.

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