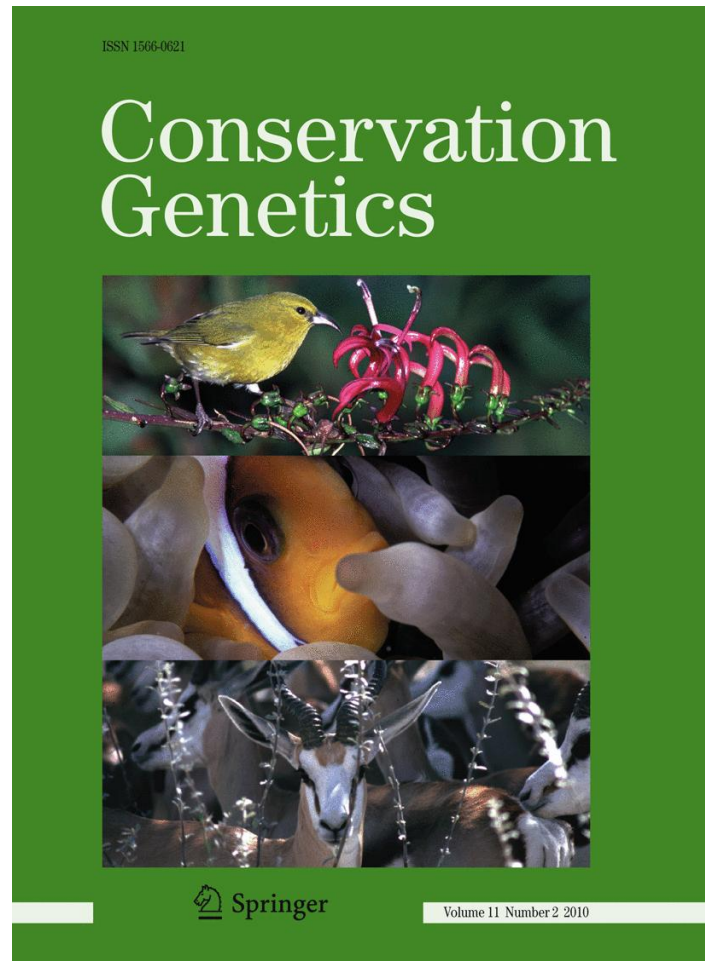


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Management of genetic diversity of subdivided populations in conservation programmes

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Abstract Population subdivision must be explicitly considered in the management of conservation programmes, as most populations of wild species at risk of extinction and those kept in captivity are spatially structured. The partition of gene and allelic diversity in within- and between-subpopulation components allows for the integral management of populations. We summarise the main aspects of this partition and some of its applications in terms of prioritisation of populations for conservation and establishment of synthetic populations. The procedures for the maintenance of diversity in subdivided populations making use of molecular markers and its implementation by the software METAPOP are illustrated with empirical data.

Keywords Inbreeding · Genetic drift · Migration · Population differentiation · Metapopulation

Introduction

Most populations of wild animal and plant species at risk of extinction are spatially fragmented. In addition, most species kept in captivity are generally maintained in independent nuclei (zoos, botanic gardens, germplasm centres, etc.) with restricted migration. The structuring of populations in reduced and sometimes isolated groups, has an

impact on the erosion of genetic variation and the increase in inbreeding, factors of paramount importance in conservation programmes (Frankham et al. 2002; Allendorf and Luikart 2007). It is, therefore, of general interest to establish methods for the management of genetic variation in subdivided populations and to look for objective criteria to quantify the importance of within- versus between-subpopulation variation.

The analysis of genetic diversity in subdivided populations is made generally in terms of gene diversity or expected heterozygosity (Nei 1973). However, allelic richness (the number of different alleles segregating in the population) is an alternative criterion for measuring genetic diversity, and some authors have considered that this parameter is of key relevance in conservation programmes (e.g. Petit et al. 1998; Notter 1999; Barker 2001; Simianer 2005; Foulley and Ollivier 2006). Allelic richness is also important from a long-term perspective, because the limit of selection response is determined by the initial number of alleles (James 1970; Hill and Rasbash 1986) and, because it is more sensitive to bottlenecks than expected heterozygosity, it reflects better past fluctuations in population size (Nei et al. 1975; Allendorf 1986; Cornuet and Luikart 1996; Luikart et al. 1998; Leberg 2002). A partition of allelic diversity in within- and between-subpopulation components can be made in an analogous way to that of gene diversity (Caballero and Rodríguez-Ramilo, unpublished), and this partition has immediate applications in the prioritisation of populations for conservation.

The control of the rate of increase in inbreeding and coancestry or, equivalently, the respective inbreeding and variance-effective population size (Caballero and Toro 2000), provide a general framework for managing genetic resources in conservation programmes (e.g. Toro and Pérez-Enciso 1990; Villanueva et al. 1994; Lacy 1995; Ballou and

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Lacy 1995; Lindgren et al. 1996; Fernández et al. 2003; Meuwissen 2007; Saura et al. 2008; Toro et al. 2009). The control of inbreeding and coancestry would restrict inbreeding depression, the probability of losing rare alleles, and the risk of extinction (Frankham et al. 2002). For single undivided populations, the consensus method for controlling inbreeding and coancestry is to optimise contributions of parents (number of offspring that each individual leaves to the next generation) by minimizing the global coancestry weighted by those contributions (Ballou and Lacy 1995). The procedure of contributions of minimum coancestry has been shown to have the following properties: (1) it maximizes the genetic diversity of the population in terms of expected heterozygosity and effective population size (Caballero and Toro 2000, 2002); (2) it is very flexible and robust against departures from the ideal conditions (Fernández et al. 2003); (3) it maintains rather efficiently the allelic richness of the population (Fernández et al. 2004); and (4) it is very effective in preserving the original distribution of allelic frequencies in conservation programmes (Saura et al. 2008). The minimum-coancestry mating system can be applied subsequently to selected individuals resulting from the optimisation (Caballero et al. 1996; Sonesson and Meuwissen 2000; Meuwissen 2007). However, algorithms are also available to optimise simultaneously the mating scheme and its contributions, the so-called mate selection (Toro and Pérez-Enciso 1990; Fernández et al. 2001).

For single undivided populations, coancestry and inbreeding change at the same rate with one preceding the other depending on the amount of non-random mating (Kimura and Crow 1963; Robertson 1964; Cockerham 1967; Caballero 1994; Wang 1997; Wang and Caballero 1999). For subdivided populations, however, overall coancestry and local inbreeding can perform differently (Wang and Caballero 1999) and a control on both will depend on the amount of migration between subpopulations. For example, it is known from classical theoretical principles, that the maximum genetic diversity of a population (the lowest overall coancestry) is attained in the long-term by subdividing it in as many isolated groups as possible (Kimura and Crow 1963; Robertson 1964; Caballero 1994; Wang and Caballero 1999), as different allelic variants will get fixed in each group, becoming a genetic reservoir of variation. However, complete isolation leads to increased rates of local inbreeding with the possible consequence of inbreeding depression. Thus, a certain degree of gene flow should be maintained through migration of individuals between subpopulations. The integral management of genetic diversity in subdivided populations under conservation programmes has not been considered until recently. Wang (2005) proposed a method to optimise the global genetic diversity controlling the rate of inbreeding from demographic information. Fernández et al.

(2008) extended this idea for the situation where genealogical information is available, by developing a flexible and dynamic methodology. This method is implemented by the software METAPOP (<http://webs.uvigo.es/anpefi/metapop/>; Pérez-Figueroa et al. 2009), which allows for the use of pedigree records or neutral molecular marker information to take conservation decisions.

In this paper, we summarise the main aspects of the partition and management of genetic diversity in subdivided populations. First, we describe the partitioning of gene and allelic diversity in subdivided populations. Second, we use empirical data to show the application of this partition on the prioritisation of populations for conservation and the establishment of synthetic populations. Finally, we illustrate the application of the dynamic method for the maintenance of diversity in subdivided populations making use of the same empirical data.

Partition of gene and allelic diversity within and between subpopulations

The total gene diversity in a subdivided population, $1 - \bar{f}$, where \bar{f} is the average coancestry (or kinship) (Malécot 1948) among all individuals of the population, can be partitioned into two components as $1 - \bar{f} = (1 - \hat{f}) + D_G$ (Nei 1973), where \hat{f} is the average of within-subpopulation coancestries and D_G is the average Nei's genetic distance between subpopulations. Assuming there is random mating (Hardy–Weinberg equilibrium) within subpopulations, \hat{f} approximates the average inbreeding coefficient (\bar{F}) within subpopulations, so that

$$1 - \bar{f} \approx (1 - \bar{F}) + D_G. \quad (1)$$

In terms of expected heterozygosities for a single locus, the above relation can be expressed as

$$H_T = H_S + D_G, \quad (2)$$

where the within-subpopulation (H_S) and the total (H_T) heterozygosities are

$$H_S = 1 - \frac{1}{n} \sum_{i=1}^n \left(\sum_{k=1}^K p_{i,k}^2 \right), \quad (3)$$

$$H_T = 1 - \sum_{k=1}^K \left(\sum_{i=1}^n \frac{p_{i,k}}{n} \right)^2 \quad (4)$$

(Nei 1973), $p_{i,k}$ is the frequency of allele k in subpopulation i , K is the total number of alleles in the population, and n is the number of subpopulations. Wright's (1969) F_{ST} is defined as the proportion of diversity between subpopulations relative to the total diversity,

$$F_{ST} = (H_T - H_S)/H_T = D_G/H_T. \tag{5}$$

An analogous partition of variation can be made regarding allelic diversity (Caballero and Rodríguez-Ramilo, unpublished). Because the number of alleles segregating for a locus in a population is highly dependent on sample size, El Mousadik and Petit (1996) proposed to estimate the number of alleles expected in samples of specified size by using the rarefaction technique (Sanders 1968; Hulbert 1971). The expected number of different alleles (the allelic richness) in a sample of genes taken at random is then equal to

$$a_i = \sum_{k=1}^K (1 - P_{ik}), \tag{6}$$

where P_{ik} is the probability that allele k does not occur in a sample of genes chosen at random with the specified sample size (El Mousadik and Petit 1996). The within-subpopulation allelic diversity can then simply be defined as the average allelic richness across subpopulations minus one (because a subpopulation with a single allele would be considered to lack variation),

$$A_S = \left(\frac{1}{n} \sum_{i=1}^n a_i \right) - 1. \tag{7}$$

An allelic dissimilarity or distance between two subpopulations may be defined as the number of alleles present in a subpopulation and absent in the other. Thus, the average allelic distance between subpopulations i and j can be obtained as

$$d_{ij} = \frac{1}{2} \sum_{k=1}^K [(1 - P_{ik})P_{jk} + P_{ik}(1 - P_{jk})], \tag{8}$$

(Weitzman 1998; Foulley and Ollivier 2006; Ollivier and Foulley 2009) and the average distance between all subpopulations is

$$D_A = \frac{1}{n^2} \left[\sum_{i,j=1}^n d_{ij} \right]. \tag{9}$$

Hence, the total allelic diversity,

$$A_T = A_S + D_A = \left[\frac{1}{n} \sum_{i=1}^n \left(a_i + \frac{1}{n} \sum_{j=1}^n d_{ij} \right) \right] - 1, \tag{10}$$

is the average pairwise diversity of subpopulations, i.e. the number of different alleles available in each pairwise grouping of subpopulations (Caballero and Rodríguez-Ramilo, unpublished). From the above expressions, a definition of the coefficient of allelic differentiation can be obtained as

$$A_{ST} = (A_T - A_S)/A_T = D_A/A_T, \tag{11}$$

which contrasts with the coefficient ρ_{ST} defined by El Mousadik and Petit (1996) (Caballero and Rodríguez-

Ramilo, unpublished). The above expressions (10–11) are analogous to the corresponding ones for gene diversity (Eqs. 2, 5) and allow for analogous applications in the prioritisation of populations for conservation and the management of subdivided populations in conservation programmes, as will be illustrated in the following section.

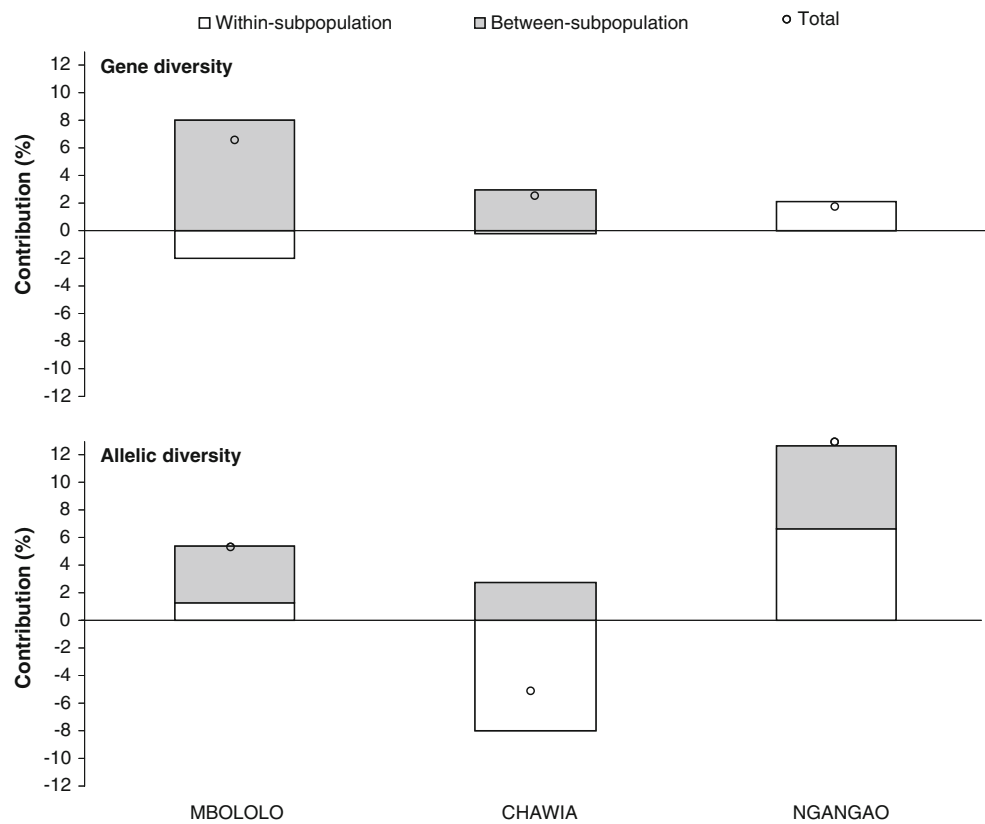
The contribution of each subpopulation to gene or allelic diversity

For a subdivided population, the contribution of each subpopulation to the total gene diversity and its components can be obtained by decomposing the two terms of expression (2) for each subpopulation, as suggested by Caballero and Toro (2002). An analogous partition can be made for allelic diversity from expression (10) (Caballero and Rodríguez-Ramilo, unpublished). An alternative method is that proposed by Petit et al. (1998), where the contribution of each subpopulation to total diversity and its components (within and between subpopulations) is estimated by disregarding that subpopulation and recalculating the global average diversity from the remaining pool. This procedure has been used both for gene diversity and allelic richness (Caballero and Toro 2002; Foulley and Ollivier 2006; Toro et al. 2009). We will illustrate this method by using empirical data on the endemic Taita thrush (*Turdus helleri*).

Galbusera et al. (2000) analysed data from seven microsatellite loci for the three remaining subpopulations of the globally, critically endangered Taita thrush in South-east Kenya. They collected data from 155 individuals from the three larger remaining forest fragments (Mbololo, sample size $N = 80$, with 29 females and 51 males; Chawia, $N = 17$, with 5 females and 12 males; and Ngangao, $N = 58$, with 25 females and 33 males), plus other tiny remnants. Whereas there were no significant differences in expected heterozygosity between the three subpopulations (0.55, 0.59 and 0.64, respectively), the allelic richness of Chawia (3.86) was somewhat lower than those of Mbololo (5.71) and Ngangao (6.14). This suggested a higher impact of moderate recent bottlenecks in Chawia, in agreement with its lower estimated effective population size (Galbusera et al. 2000). The gene diversity differentiation (F_{ST}) among subpopulations was around 0.2, the Mbololo subpopulation showing a somewhat higher average genetic differentiation with the others.

Because the current METAPOPOP software does not allow for sex-linked genes, we used data for the six autosomal loci to estimate the contribution of each subpopulation to gene and allelic diversity. The data is available in <http://fisher.berkeley.edu/structurama/>.

Fig. 1 Proportional contribution to gene or allelic diversity from each of the three subpopulations of Taita thrush in south-east Kenya (Galbusera et al. 2000). Positive values indicate a loss of diversity when the subpopulation is removed from the population, and vice versa. Circles indicate the contribution to overall gene/allelic diversity, white boxes and dark boxes give the contribution to within- and between-subpopulation diversity, respectively



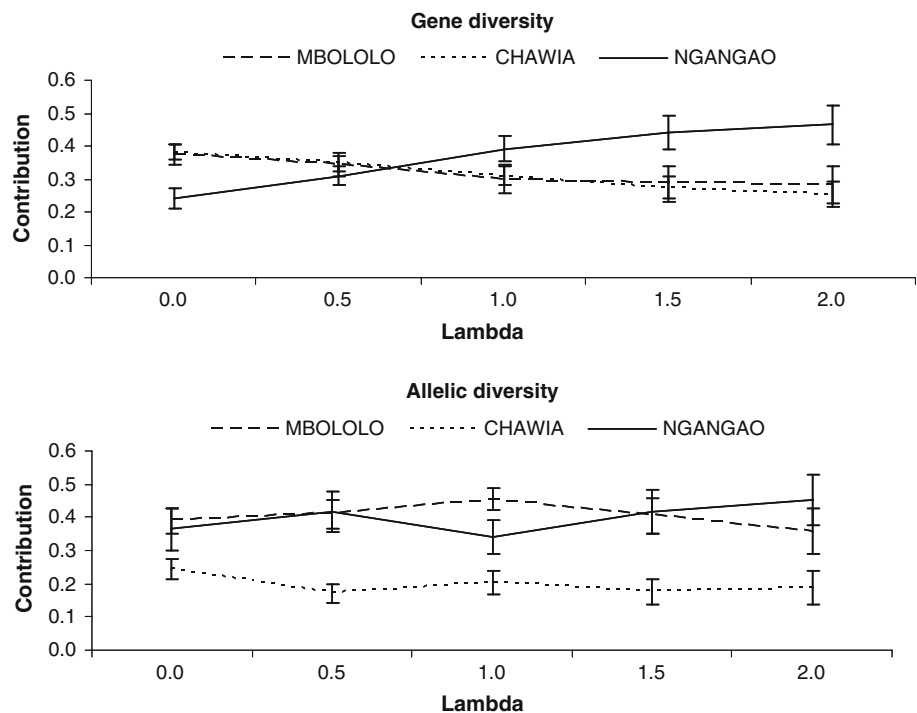
In Fig. 1, the contribution of each subpopulation to gene and allelic diversity is quantified as the percentage of gene/allelic diversity lost (positive sign) or gained (negative sign) with the removal of the subpopulation. Allelic diversity measures were obtained after rarefaction assuming the minimum sample size of Chawia (34 genes). The highest contribution to gene diversity was that of Mbololo, because of its higher contribution to the between-subpopulation component of variation, and despite its negative contribution to the within-subpopulation component. The lowest contribution to gene diversity was that of Ngangao. This presented the highest contribution to within-subpopulation variation but a null contribution to the between-subpopulation component. In contrast, for allelic diversity, Ngangao had the largest contribution to diversity (both to within- and between-subpopulation components). Chawia had the lowest contribution, particularly because of its negative contribution to the within-subpopulation component. This fully agrees with the lower allelic richness found in Chawia and the fact that only two private alleles were found in this subpopulation, whereas eight private alleles were found in each of the other two. These results illustrate that conservation decisions based on gene and allelic diversity may differ substantially and will depend on the emphasis given to the two measures of diversity.

The contribution of each subpopulation to a synthetic population

The contribution of each subpopulation to a synthetic population or gene pool of maximum gene or allelic diversity can also be a way to prioritise subpopulations for conservation (Caballero and Toro 2002; Eding et al. 2002; Ollivier and Foulley 2005) and can be useful when germplasm stocks are created. These optimal contributions can be applied considering a weighted combination of the within- and between-subpopulation components of gene diversity, where a factor λ can be used to give the desired weight to the within-subpopulation component. In the case of gene diversity, the two terms are those of expression (2), i.e. $\lambda H_S + D_G$, whereas for allelic diversity they would be those of expression (10), i.e. $\lambda A_S + D_A$. The proportional contributions of the three subpopulations of Taita thrush for different weighting factors are given in Fig. 2 (optimal solutions were obtained via *simulated annealing* algorithms; see, e.g. Fernández et al. 2003). Because the analysis was made for each locus separately, there is a certain variation in the solutions. The figure shows the optimal contributions averaged over loci with a bar denoting the standard error of the mean.

Some discrepancies can be noted between the expected contribution of each subpopulation to the pool regarding

Fig. 2 Relative contributions of each subpopulation of Taita thrush to a pool of maximal gene diversity or allelic diversity. The function optimised is $\lambda H_S + D_G$ (for gene diversity) and $\lambda A_S + D_A$ (for allelic diversity) (see text), where λ is the weight given to within-subpopulation diversity. The contributions are calculated for each locus separately and averaged, the bar denoting one standard error of the mean



gene or allelic diversity. In the case of gene diversity, if $\lambda = 0$, i.e. between-subpopulation gene diversity is the only optimising criterion, Mbololo and Chawia would have the largest contributions (around 40% each) whereas Ngangao would contribute 20% to the pool. In contrast, if $\lambda = 2$, i.e. within-subpopulation allelic diversity is given more importance as the optimising criterion, Ngangao would have the largest contribution (about 50%) and the other subpopulations would contribute about 25% each. For allelic diversity, however, the relative contributions would change little with the value of λ , Chawia giving always the lowest contribution (about 20%) and the others about 40% each.

Management of gene and allelic diversity in subdivided populations

In its original formulation, the method developed by Fernández et al. (2008) is aimed at looking for the optimal contributions from each individual so as to maximise total gene diversity with a given restriction (if desired) in the rate of increase in local inbreeding and the maximum number of migrations allowed among subpopulations. The method also provides the optimum translocations between subpopulations so as to achieve the highest overall gene diversity. This method is implemented by the software METAPOP (Pérez-Figueroa et al. 2009), the latest version of which includes several

developments in addition to those presented in the original model of Fernández et al. (2008). First, a mate selection strategy is applied (see Fernández et al. 2001, and references therein), so that the optimisation algorithm provides not only the optimal number of offspring contributed by a given parent but also the particular couple to which it should be mated so as to perform minimum coancestry mating. Second, management criteria can be based on allelic diversity, as described above, rather than on gene diversity.

Because the METAPOP output provides the coancestry matrix among the expected progeny, this matrix can be directly used as input data in order to rerun the program and find the contributions of individuals and migrations expected in a further generation. This is useful to simulate the expected outcome of the method if applied over generations. We used this approach to see the expected impact of the Dynamic method in comparison with other alternatives using the Taita thrush data. Because the purpose of the analysis was only illustrative, we assumed that the subpopulation sizes were those given by the sample sizes, and these sizes and the corresponding sex ratios were maintained as constants through 5 generations of breeding in controlled conditions. We considered four scenarios: (1) *NM* (no management): The three subpopulations were maintained without management assuming polygamous random mating within subpopulations, and random contributions from parents to progeny. No migration was allowed between subpopulations. (2) *NM + M* (no

management with migration): The same as the previous method except that one migrant per generation and subpopulation was allowed between subpopulations. In particular, and for the sake of simplicity, every generation one female was randomly chosen from Mbololo and translocated to Chawia, one random female from Chawia was translocated to Ngangao, and one random female from Ngangao was translocated to Mbololo. Females were translocated instead of males because of the male-biased ratio in all subpopulations. The two above methods were simulated with a computer programme written in C. (3) *MC + M* (minimum coancestry mating and contributions within subpopulations, with random migration): The software METAPOP was used to run the One-Migrant-Per-Generation strategy (OMPG) option. Each subpopulation was managed separately. Numbers of progeny contributed by parents and mating design within subpopulations were optimised following either the minimum coancestry (maximum gene diversity, H_S) criterion (Ballou and Lacy 1995) or the maximum allelic diversity (A_S) criterion (Fernández et al. 2004). In each generation every subpopulation sent a descendant and received a descendant from another randomly chosen subpopulation. (4) *DM* (Dynamic method): The software METAPOP was also used to run the Dynamic method. Mating pairs and their contributions to progeny, numbers of migrants and their subpopulations of origin and destiny were optimised following the model developed by Fernández et al. (2008). For the sake of a fair comparison with the *MC + M* and *NM + M* methods, the maximum average number of migrants allowed per subpopulation and generation was one. The optimisation criterion followed was to establish contributions that maximise the overall population gene diversity (H_T) or the total allelic diversity (A_T). Note that the difference between *MC + M* and *DM* is that, for the former, contributions of minimum coancestry are optimised for each subpopulation separately and migrations are random, whereas for the latter, contributions of minimum coancestry are optimised for the whole population and migrations are arranged from the optimisation procedure. For all methods, five replicates were run and the results were averaged over replicates.

Figure 3 shows simulated values of total (H_T) and within-subpopulation (H_S) gene diversities, and total (A_T) and within-subpopulation allelic diversities (A_S), as well as the average total number of alleles (K) for the four methods considered. All estimates of variation declined for the methods with no management (*NM* and *NM + M*), although the decline was smaller when migration was allowed between subpopulations (*NM + M*). The management methods implied an increase in the estimates of variation and the maintenance of the initial number of alleles (K). The *DM* method produced generally better results than the *MC + M* method. The exception was for

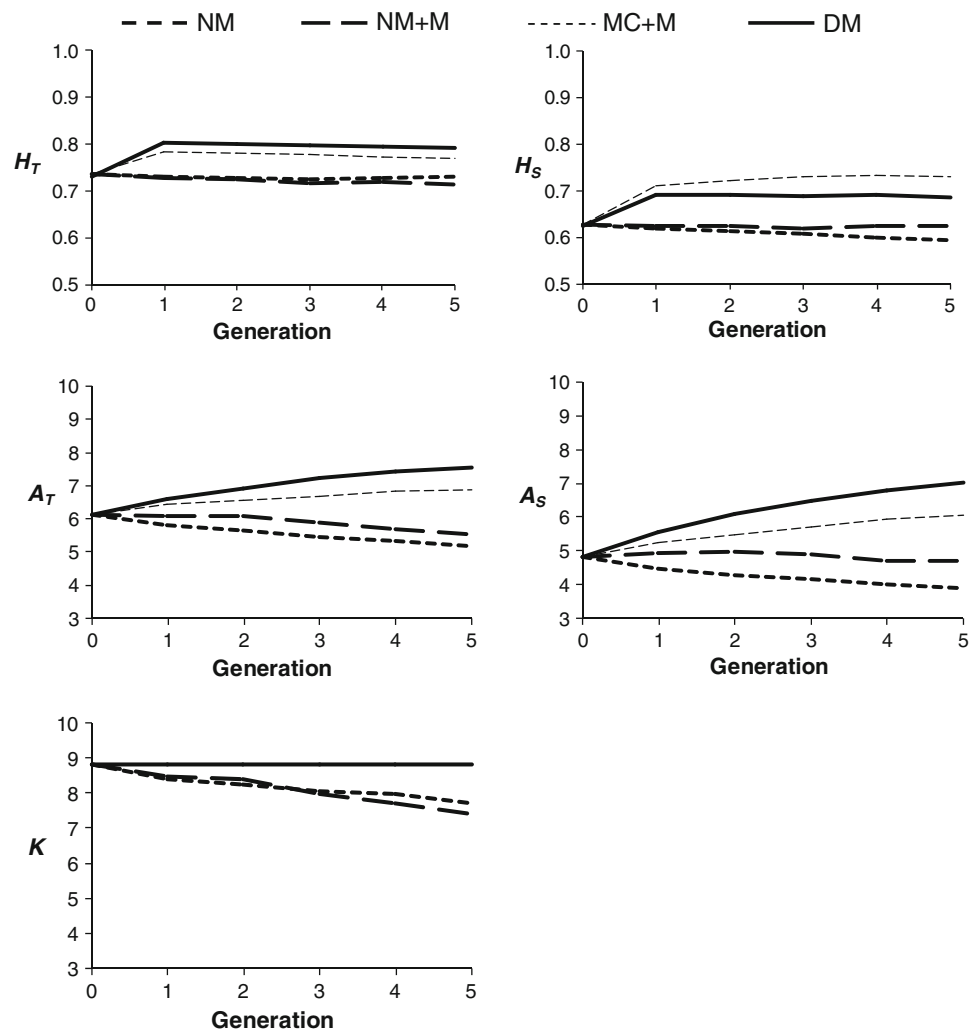
H_S . The reason could be that in the *MC + M* method the optimisation procedure to minimise the coancestry took place within each subpopulation, whereas in the *DM* the minimum coancestry optimisation was directed towards the whole population.

Discussion

Most, if not all, populations of species under the threat of extinction are subdivided, because habitat fragmentation is one of the most common outcomes of the environmental changes induced by human activities (Frankham et al. 2002). In addition, most populations of species maintained in captivity for conservation purposes are subdivided, mainly because of practical reasons (Margan et al. 1998). Thus, methods of analysis and management of genetic variation in subdivided populations are greatly needed.

The majority of analyses of genetic variation in studies for conservation purposes are focused on gene diversity (i.e. expected heterozygosity) and its partition in within- and between-subpopulation components. The analysis of the partition of allelic variation is less common, although it has been often applied in plant studies (e.g. Petit et al. 1998; Comps et al. 2001; Tyler 2002; Persson et al. 2004; Stefenon et al. 2008). Allelic richness is a measure of genetic variation somehow different from gene diversity. Situations can be given of populations with the same heterozygosity but different allelic richness and vice versa. For instance, a population with two alleles with frequencies of 0.5 each has the same expected heterozygosity (0.5) as a population with 5 alleles with frequencies of 0.69, 0.08, 0.08, 0.08 and 0.07, and the same as another with 10 alleles with frequencies of 0.7, 0.04 (6 alleles) and 0.02 (3 alleles). However, the consequences of these different population compositions can be different in terms of the potentiality of the population for adaptation and evolution. Because the short-term response and inbreeding depression depend directly on expected heterozygosity (gene diversity), these will be the same for the above situations with different allelic numbers and the same heterozygosity. However, the long-term selection will be potentially higher in populations with larger allelic diversity. Allelic richness is, in addition, more sensitive to demographic changes (Allendorf 1986; Luikart et al. 1998) and selective sweeps (Santiago and Caballero 1998) than heterozygosity. This argument could be directly extended to structured populations, suggesting that allelic differentiation must be a complementary parameter to gene frequency differentiation regarding the potential of natural populations for adaptation and the criteria to be applied for the management of conserved populations. The partition of gene and allelic diversity into their components may have direct

Fig. 3 Estimates of total (H_T) and within-subpopulation (H_S) gene diversities, total (A_T) and within-subpopulation (A_S) allelic diversities and average total number of alleles (K) over five generations. Four breeding methods were compared: *NM* no management of subpopulations, which were kept isolated. *NM + M*: no management with one migrant per generation and subpopulation. *MC + M*: optimisation of contributions from parents to progeny to achieve the minimum coancestry (maximum allelic richness) in each subpopulation, allowing for one random migrant per generation and subpopulation. *DM*: Dynamic method; optimisation of contributions from parents to progeny and migrations to achieve the minimum coancestry in the whole population (maximum total allelic diversity), with a maximum global number of migrants equal to that for *MC + M*



applications in conservation settings. First, in the analysis of the contribution of each subpopulation to the genetic variation harboured by the whole population; second, in the establishment of synthetic populations, such as in the case of the creation of germ-plasm collections; and, finally, in the management of populations in captive breeding schemes.

The relative contribution of each subpopulation to genetic diversity can be calculated both for gene and allelic diversity by disregarding the subpopulation and working out the gain or loss in diversity after the removal (Petit et al. 1998). The partition of allelic diversity proposed by Caballero and Rodríguez-Ramilo (unpublished) and described in this paper differs from a previous one for allelic richness proposed by Petit et al. (1998). Basically, for this latter method, only subpopulations with private alleles contribute substantially to the whole allelic richness (Petit et al. 1998; Foulley and Ollivier 2006; Toro et al. 2009; Ollivier and Foulley 2009), whereas the method presented here considers the allelic differences

between subpopulations, arising both from private and common alleles (Caballero and Rodríguez-Ramilo, unpublished).

By removing one subpopulation and recalculating the genetic diversity it is possible to envisage the impact of a possible extinction of the subpopulation on the system, with consequences for conservation decisions. The application of the partition of gene and allelic diversity to the Taita thrush data clearly shows that these decisions might be radically different if based on one or the other criterion. The subpopulation with the highest overall contribution to gene diversity is the Mbololo subpopulation, basically because of its high gene frequency differentiation with the others (Fig. 1). In contrast, the Ngangao subpopulation is that with the highest overall contribution to allelic diversity, both because of its high within-subpopulation allelic richness and its high allelic differentiation with the others. Note that allelic diversity calculations are made after rarefaction is applied, i.e. they account for the different sample sizes of the three subpopulations. Therefore,

conservation priorities would be different under the two criteria. The Ngangao subpopulation has a higher allelic richness, a higher heterozygosity, and a higher average allelic differentiation with the other subpopulations than the Mbololo subpopulation. However, it has a lower overall gene frequency differentiation with the other subpopulations than the Mbololo subpopulation. From a gene frequency perspective, the Mbololo subpopulation is the most valuable, whereas in terms of allelic diversity, it is the Ngangao subpopulation the most valuable.

The decision about which of the measures of diversity should be given more importance is not straightforward, and it likely depends on the particular situation of the population and the conservation objectives. As mentioned above, the amount of inbreeding depression and the immediate response to selection depend on the expected heterozygosity, i.e. on gene diversity. Therefore, a short-term conservation effort in a population under high risk of extinction should probably be devoted to maintaining the highest levels of gene diversity. In contrast, the long-term response to selection and future capability of adaptation depend largely on the allelic resources available in the population. Thus, a population whose viability is not under imminent risk could probably be targeted towards maintaining the largest possible allelic diversity. This could be particularly appropriate for a structured population which shows particular adaptations in different subpopulations, making important to maintain the alleles responsible for the adaptation. The definition of short term or long term is, however, arbitrary, as it depends on several factors, mainly the variation of allelic effects and the intensity of selection. Obviously, the two measures of diversity are related with each other and it may be possible to combine both sources of information to reach global optimal solutions. This clearly requires further research.

Toro et al. (2009) also proposed an alternative way for the partition of genetic diversity which, to a certain extent, takes allelic diversity into consideration. Because the larger the number of alleles, the larger is the potential diversity of a subpopulation, and because the maximal diversity occurs when alleles are at equal frequencies, a possible strategy could be to look for contributions based on the maximal attainable diversity, i.e. in a situation where all alleles present in a subpopulation have identical frequencies. Thus, the method proposed by Toro et al. (2009) consists of analysing gene diversity in the standard way (Eqs. 1–5) but assuming that all alleles in each subpopulation have the same frequency. An estimate of gene diversity under this situation accounts, at least partially, for the allelic diversity of the population by considering the potentiality of each subpopulation according to the number and type of alleles that it carries. Because maximising gene diversity using molecular markers leads to equal allele frequencies (Saura

et al. 2008), the assumption of this method is that the subpopulations have achieved the highest possible gene diversity with all alleles initially available. This procedure, in fact, leads to the maximisation of the effective number of alleles (Crow and Kimura 1970, p. 324), defined as the number of alleles with equal frequencies that would yield an expected heterozygosity equal to the observed one. Therefore, the contributions from each subpopulation would refer to a hypothetical situation in which these have been subjected to an efficient conservation programme where all alleles initially present have been preserved and led to equal frequencies. The application of this method to the Taita thrush example gives results somewhat different from those given in Figs. 1 and 2. For example, the highest contribution to diversity is about the same for Mbololo and Ngangao subpopulations, with Chawia contributing less. In this respect, the result is a consensus of the previous ones (Mbololo contributed with the highest gene diversity and Ngangao with the highest allelic diversity; Fig. 1). The contributions to a synthetic population using the Toro et al. (2009) approach are closer to those of gene diversity for $\lambda = 0$ (Fig. 2), closer to those of allelic diversity for $\lambda = 1$, but different from any of them for $\lambda = 2$, with Mbololo ranked first, then Ngangao and finally Chawia. Nevertheless, because this procedure refers to a hypothetical situation of highest gene diversity rather than to the current situation of the population, its interpretation is complicated.

Another way to prioritise subpopulations is to calculate the contribution of each one to a pool of individuals or a germplasm bank that would maximise its genetic diversity (Bataillon et al. 1996; Caballero and Toro 2002; Eding et al. 2002). The partition in within- and between-subpopulation components allows for a specific desired weight (λ) to be applied in the optimising function, i.e. $\lambda H_S + D_G$ (for gene diversity) and $\lambda A_S + D_A$ (for allelic diversity). The use of gene or allelic diversity criteria in the above partition can again generate different outcomes, as illustrated with the Taita thrush example in Fig. 2. From the gene diversity perspective the Ngangao subpopulation contributes increasingly more as λ is increased, i.e. more weight is given to the within-subpopulation, whereas the two other subpopulations have the opposite trend. This is generally consistent with the proportional contributions shown in Fig. 1, but note that these are different ways of prioritisation and they do not have to give identical results. From the allelic diversity point of view it becomes clear that the Chawia subpopulation is that which is contributing less, whatever the value of λ considered. A way of combining the two criteria should clearly be pursued in future work.

The value of λ to be applied for the creation of a synthetic population will depend on the particular interests for

each situation, and several alternatives have been proposed so far regarding gene diversity. For example, a value $\lambda = 1$ (equal weight to within- and between-group components) is analogous to weighting the between-subpopulation component by F_{ST} and the within-subpopulation component by $1 - F_{ST}$ (Ollivier and Foulley 2005). Piyasatian and Kinghorn (2003) suggested giving five times more weight to the variation between subpopulations than to that within subpopulations ($\lambda = 0.2$). Bennewitz and Meuwissen (2005) also proposed an automatic weight based on maximizing the total genetic variance of a hypothetical quantitative trait, which is equivalent to using a weighting factor of $\lambda = 0.5$. Again, the election of the particular λ to be applied in a conservation programme must depend on the particular situation. In general, if it is desired that the peculiarities of the rare alleles present in some subpopulation should be preserved in the synthetic population, more weight should be given to λ . In contrast, if a more uniform set is desired where only alleles with substantial frequencies are maintained, for example to obtain a high short-term selection response, less weight should be given to λ .

The management of genetic diversity in conservation programmes is rather straightforward in single undivided populations (Ballou and Lacy 1995; Meuwissen 2007; Toro et al. 2009), with the simple objective of controlling the coancestry or inbreeding of the population, which behave in parallel in a single population. In a subdivided population, however, there is a conflict between overall coancestry (\bar{f}) (or total diversity H_T) and local inbreeding (\bar{F}) (or within-subpopulation diversity H_S), as expressed in Eqs. 1–2. Complete isolation of subpopulations generates the lowest overall coancestry (the highest H_T) in the long term at the cost of an increase in the genetic distance between subpopulations (D_G) and an increase in the local inbreeding (decrease in H_S). As migration takes place, D_G becomes reduced and H_S increased, implying a reduction in total overall diversity (H_T). Therefore, if the aim is to increase the overall diversity while controlling local inbreeding, the migration rate must be tuned so as to achieve both goals. Beyond this, another issue to account for in the management of subdivided populations is the fact that subpopulations may be locally adapted and have evolved differentially. Consequently, some degree of differentiation/separation would be desired and the mixing of subpopulations should be carefully planned. The Dynamic method developed by Fernández et al. (2008) is aimed at accounting for this conflict, by looking for the contributions of parents and the particular migrations that maintain the largest possible genetic diversity in the population controlling for local inbreeding.

The example of the Taita thrush data shows that the optimisation of contributions from parents to progeny

(MC + M and DM methods) implies an increase over generations of all measures of diversity, as well as the maintenance of all the alleles present initially in the population (Fig. 3). The particular optimised migration scheme provided by the Dynamic method generally overcomes others where optimisations of contributions are made within subpopulations and migrations are randomly arranged (MC + M method). The superiority of the Dynamic method over this latter one was shown in simulations by Fernández et al. (2008) and also experimentally with *Drosophila melanogaster* by Ávila et al. (unpublished). When optimal contributions are not sought (NM and NM + M), all estimates of diversity decline over generations, including a clear loss of total number of alleles present in the population. Random migration between subpopulations produces a slight benefit but this is not great (cf. NM and NM + M). Thus, the possibility of optimising parents' contributions and also the migration patterns clearly implies an efficient management of genetic diversity. Of course, the example shown in Fig. 3 was given for the purpose of illustration. It is highly unlikely that such detailed management could be ever carried out with the Taita thrush populations or similar wild populations. However, the method can be applied, at least partially, in subdivided populations of species maintained in captivity under the possibility of a more controlled manipulation (e.g. zoo populations).

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