

Genetic Diversity Management of Moroccan Captive-Bred Houbaras

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Abstract

With regards to an *ex-situ* conservation plan and program of Moroccan houbara bustards, the genetic diversity of a captive breeding stock of (*Chamidotis undulata undulata*) was studied and assessed using metapopulation approaches. The present study aims thus, the description and comparison of various strategies implemented in the species conservation that would conduct to: 1) a better quantification of the gain and loss of genetic diversity of the houbara herd made up of wild and captive populations, and consequently, to 2) a pertinent tracing of conservation and management priorities of the Moroccan avian subspecies.

Keywords

Ex-situ Conservation, Genetic Resource Management, Moroccan Houbara

1. Introduction

Bustard Houbara is an avian species classified as vulnerable by the International Union for Conservation of Nature (IUCN) in 2016. It belongs to the *Otididae* family and is banned from international trade by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Since 1993, the Houbara bustard has been bred in captivity by the Prince Sultan Bin Abdul Aziz Al Saud Foundation (IFCDW) in Agadir, Morocco, and several conservation methods were developed. Generally, maintaining and preserving genetic diversity is essential in this type of program, as the loss and deterioration of

genetic diversity and increased inbreeding may lead to inbreeding depression, bottlenecks, non-adaptation to environmental change, and consequently extinction of species and populations [1].

In addition to the technological advances in molecular biology and bioinformatics, a wide variety of genetic and statistical recommendations and approaches have been proposed for the study of genetic diversity in the conservation context [2] [3]. These techniques are generally based on the measurement of gene diversity, also known as “heterozygosity” [4] and/or allelic richness [5] [6]. In 1992, Weitzman [7] used phylogenetic methods based on genetic distances in the analysis of global genetic diversity to determine conservation priorities. However, this approach has been criticized because it did not take into account within (sub)population diversity in its estimation [8].

More recently, to study genetic diversity extracted either from genealogies (pedigrees) or neutral molecular markers such as microsatellites, the most plausible and accepted strategy could be the one aimed at minimizing the kinship or coancestry in a metapopulation, by optimizing and maximizing the contribution of parents to subsequent generations [3]. In the case of subdivided or poorly structured populations, it was shown that this practice works in favor of genetic diversity by increasing the expected heterozygosity and the effective size of a population [9].

Furthermore, among the causes of decline of genetic diversity, is the reduction of the effective size of a population (N_e) which is characterized by a decline in the number of alleles, and can in the long term, influence the species survival.

The aim of this research is to describe and compare different approaches that measure genetic diversity in terms of expected heterozygosity, mean coancestry and allelic richness with rarefaction, as well as to better assess the gain and loss of genetic diversity of a Moroccan Houbara bustard herd of recent pedigree, which is made up of wild founder and captive populations. Such study is believed to enable setting priorities for the conservation and management of the Moroccan avian subspecies.

2. Materials and Methods

2.1. Samples

A total of 799 birds belonging to 5 different populations were used for this study (Table 1). The genealogy of the 4 captive-born individuals of the population is made available through molecular paternity service provided by the IFCDW Genetics Department [10].

2.2. Molecular Analysis

Genomic DNA was extracted from blood samples and genotyped by Polymerase Chain Reaction (PCR), using 4 pairs of polymorphic primers: A210, D117, D110 and A205 as described elsewhere [11]. The revelation of the PCR products was carried out by the Li-COR 4000L automatic sequencer.

Table 1. Numbers, origins and dates of collection of the five Houbara bustard populations studied ($n = 799$). (The source of all the samples: IFCDW).

Subpopulations	Number	Origin	Date of sampling
Subpopulation 1	25	Wild from Erfoud	1993-1994
Subpopulation 2	21	Wild from Boudnib	1993-1994
Subpopulation 3	89	Wild from Errachidia	1993-1994
Subpopulation 4	562	Captive and issued from populations 1, 2 and 3	1995-2004
Subpopulation 5 (CO6)	102	Wild from the East of Morocco (Towards Algeria)	2006

2.3. Statistical Analysis

2.3.1. Measurement of Genetic Diversity in Terms of Expected Gene Diversity or Heterozygosity

Total heterozygosity (H_T) and allele frequencies were calculated using Arlequin v.3.5.1.2 [12] and GenAlex v.6.3 [13] programs. H_T is partitioned into between population gene diversity D_{ST} and within population gene diversity H_S ($H_T = D_{ST} + H_S$). It is also equal to the expected total heterozygosity defined as:

$$H_T = \frac{2n}{2(n-1)} \left(1 - \sum_{i=1}^n p_i^2 \right),$$

with the mean p_i allele frequencies of the i th allele

in all populations or subpopulations studied. The effective number of alleles ($N_e = \frac{1}{\sum_{i=1}^n p_i^2}$) [14] and Shannon's index of diversity or information [15]

$$(I = -\sum_{i=1}^n p_i \ln(p_i))$$

were also estimated by GenAlex [13]. Micro-Checker 2.2.3 [16] was used to identify genotyping errors. The deviation from Hardy-Weinberg equilibrium for each population was investigated by Fisher's exact test, using GENEPOP software [17] and considering 50,000 iterations and 20 batches.

The analysis of molecular variance (AMOVA) performed by Arlequin facilitated the quantification of the hierarchical structuring of genetic variance at within and between populations (or subpopulation) levels. The same program estimated genetic differentiation F_{ST} and its probability value (P -value) between different pairs of populations.

2.3.2. Measurement of Genetic Diversity in Terms of Mean Coancestry f_{ij}

According to Malécot [18], the coancestry coefficient (f_{ij}) between two individuals i and j , is the probability that two alleles at a locus taken at random are identical. Furthermore, the total genetic diversity (DG_T) in a particular subdivided population or metapopulation, corresponds to the complement of the global coancestry coefficient ($DG_T = 1 - \bar{f}$).

The theories of Caballero and Toro [8] are applied both to genealogical data and to those generated by neutral molecular markers, and the only requirement

between the two approaches is that the coefficient (f_{ij}) between all pairs of individuals should be calculated by considering “identity by descent” in the case of pedigrees [18], and considering “identity in state” in the case of neutral markers.

In general, considering a metapopulation composed of n populations or subpopulations of N_i individuals, the average coefficient of inbreeding of the subpopulation i is: ($F_i = 2s_i - 1$) with s_i the average self coancestry of all the individuals. Also, the average distance between the individuals belonging to subpopulations i and j is defined as $D_{ij} = \left[\frac{(s_i + s_j)}{2} \right] - f_{ij}$. As for the minimum

distance of Nei (1987) it is calculated by applying the equation:

$D_{ij(Nei)} = D_{ij} - \left[\frac{(D_{ii} + D_{jj})}{2} \right] = \left[\frac{(f_{ii} + f_{jj})}{2} \right] - f_{ij}$, with (f_{ii}) and (f_{jj}) the average coancestries, (D_{ii}) and (D_{jj}) of subpopulations I and J , respectively.

Furthermore, at the within subpopulation level, the average coefficient of coancestry is expressed as: $\tilde{f} = \frac{\sum_{i=1}^n f_{ii} N_i}{N_T}$, the average of self-coancestry as:

$\tilde{s} = \frac{\sum_{i=1}^n s_i N_i}{N_T}$, the average inbreeding coefficient as: $\tilde{F} = \frac{\sum_{i=1}^n F_i N_i}{N_T}$, and the average

distance between individuals as: $\tilde{D} = \tilde{s} - \tilde{f} = \frac{\sum_{i=1}^n D_{ii} N_i}{N_T}$ with, $N_T = \sum_{i=1}^n N_i$ as the overall size of the metapopulation. Consequently, the average coancestry and genetic distance over the entire metapopulation are:

$$\bar{f} = \tilde{f} - \bar{D} = \frac{\sum_{i=1}^n f_{ii} N_i}{N_T} - \bar{D} = \frac{\sum_{i,j=1}^n f_{ij} N_i N_j}{N_T^2} = \sum_{i=1}^n \frac{N_i}{N_T} \left[f_{ii} - \frac{\sum_{j=1}^n D_{ij} N_j}{N_T} \right] \text{ and}$$

$$\bar{D} = \frac{\sum_{i,j=1}^n D_{ij} N_i N_j}{N_T^2}, \text{ respectively. Total gene diversity } (DG_T = 1 - \bar{f}) \text{ is thus, com-}$$

posed of between subpopulation diversity ($DG_{BS} = \tilde{f} - \bar{f}$) and within subpopulation diversity ($DG_{WS} = 1 - \tilde{f}$). The latter is the sum of within individual diversity ($DG_{WI} = 1 - \tilde{s}$) and among individual diversity ($DG_{BI} = 1 - \tilde{s}$). The contributions of subpopulations i and j to the next generation can be extracted from the total gene diversity by applying the following equation:

$$DG_T = 1 - \bar{f} = 1 - \sum_{i,j=1}^n f_{ij} c_i c_j = 1 - \sum_{i=1}^n c_i \left(f_{ii} - \sum_{j=1}^n D_{ij} c_j \right).$$

Finally, Wright’s coefficient [19] of differentiation is calculated using the following formula:

$$F_{ST} = \frac{GD_{BS}}{GD_T} = \frac{\tilde{f} - \bar{f}}{1 - \bar{f}}.$$

The aforementioned analyses were carried out using the METAPOPOP v.2.0.a3

software [20], while the calculation of the mean meta-population matching was made using the MOLKIN v.3.0 program [21].

2.3.3. Measurement of Genetic Diversity in Terms of Allelic Richness with Rarefaction

The allelic richness or the number of alleles per locus is estimated based on the classical rarefaction method proposed by El Mousadik and Petit [22] and Petit *et al.* [5]. This method makes it possible to correct the bias resulting from the differences in sample sizes by considering the number of expected alleles of a sample whose size (g) is smaller than that of a larger size (N_i). N_i represents the total number of genes in a population (i) and N_{ik} is the number of copies of the k^{th} allele of a sample belonging to a given population. The allelic richness in a

$$\text{given locus is: } A_i = \sum_{k=1}^K (1 - P_{ik}) \quad \text{with, } P_{ik} = \frac{(N_i - N_{ik})}{\binom{N_i}{g}}.$$

By analogy with the coefficient of genetic differentiation F_{ST} , a coefficient of differentiation of allelic richness was also proposed by El Mousadik and Petit [22]: $\rho_{ST} = \frac{R_T - R_S}{R_T - 1}$ with, ($R_S = \frac{1}{n} \sum_{i=1}^n a_i$) the mean within population allelic richness, R_T the total allelic richness, and a_i the number of alleles.

The new methodology based on the principle of rarefaction and the partition of allelic richness into within and between population diversity and proposed by Caballero and Rodríguez-Ramilo [23], was applied in this work in order to compare the two approaches with rarefaction. In this case, within population allelic diversity is calculated as: $A_S = R_S - 1 = \left[\frac{1}{n} \sum_{i=1}^n a_i \right] - 1$, while the mean allelic distance between populations i is calculated as:

$$d_{A,ij} = \frac{1}{2} \sum_{k=1}^K [(1 - P_{ik})P_{jk} + P_{ik}(1 - P_{jk})]. \quad \text{The average distance between all the populations is thus equal to: } D_A = \frac{1}{n^2} \left(\sum_{i,j=1}^n d_{A,ij} \right).$$

$$\text{is given as: } A_T = A_S + D_A = \left[\frac{1}{n} \sum_{i=1}^n \left(a_i + \frac{1}{n} \sum_{j=1}^n d_{ij} \right) \right] - 1 = \left[\frac{1}{n^2} \sum_{k=1}^K \sum_{i,j=1}^n (1 - P_{ik}P_{jk}) \right] - 1.$$

The contribution of each population to total genetic diversity can also be deduced from this equation as well as the coefficient of allelic differentiation which is equal to: $A_{ST} = \frac{D_A}{A_T} = \frac{A_T - A_S}{A_T}$.

2.3.4. Contribution of Each (Sub)population to the Total Genetic Diversity

The method of Kirkpatrick *et al.* [24] based on the simulated annealing logarithm, was used to rank the different populations according to their optimal contributions to an artificial gene pool (germplasm) possessing maximum genetic diversity. According to this approach, optimal contributions can be applied

by considering a population factor λ which, depending on the interest and conservation strategy, gives weight and importance to intra- and/or inter-population gene diversity ($H_T = \lambda H_S + D_{ST}$) [25]. All these analyses were carried out using the METAPOPOP software [20].

3. Results

3.1. Gene Diversity and Genetic Differentiation

The Fisher’s exact test revealed no linkage disequilibrium between the different loci. Micro-checker software also did not detect any signs of marginalization of large alleles, genotyping and profiling errors or the existence of null alleles. The amplification of the four microsatellites generated 28 alleles in total for the 799 individuals studied. No private or rare alleles were detected in the wild populations of Erfoud, Errachidia, Boudnib or the captive population of 1995-2004. On the other hand, among the 2006 wild population (CO6), 3 alleles (162) were revealed at the locus D117 (corresponding to an allelic frequency of 0.015) and 8 alleles (233) at the A205 locus (corresponding to an allelic frequency of 0.039) (Figure 1). The detailed allelic structure of each population is presented in Figure 2.

In Table 2, the global genetic diversity H_e value was estimated at 0.686, and the within-subpopulation expected heterozygosity (H_e) was found to be high in the founder and wild populations of Errachidia, Boudnib and CO6, comparing to the captive subpopulation (0.694, 0.691, and 0.681 vs 0.656, respectively). This

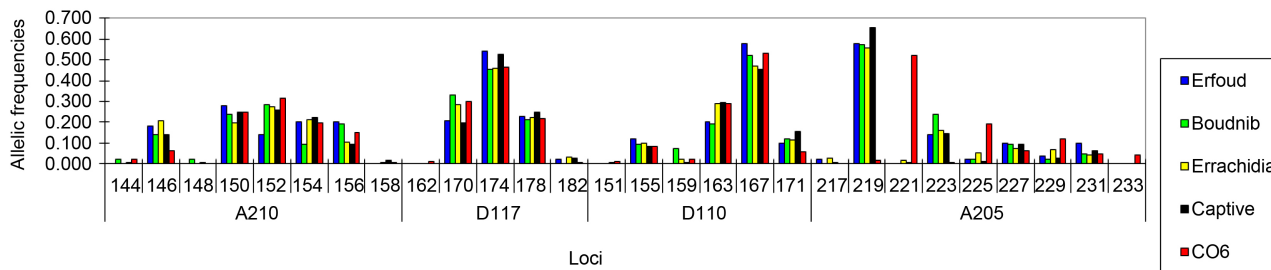


Figure 1. Distribution of alleles and their frequencies for each locus studied and within the five studied subpopulations. (N. B: For the D110 locus, the 151 allele was detected in the CO6 population and captive from 1995-2004 but at a very low allelic frequency, e.g.: (0.003) for the latter).

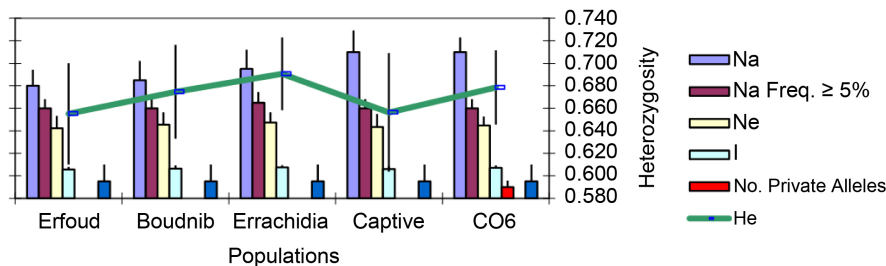


Figure 2. Allelic structure of each population. Na: number of alleles, Ne: number of effective alleles, I: Shannon index, He: Expected heterozygosity. (In red the number of rare alleles).

Table 2. Global genetic diversity within each population and by locus.

Loci	Erfoud	Boudnib	Errachidia	Captive	CO6	Het. Total
A210	0.8057	0.8141	0.7915	0.7922	0.7769	0.7930
D117	0.6232	0.6538	0.6609	0.6217	0.6501	0.6322
D110	0.6114	0.6771	0.6738	0.6740	0.6265	0.6670
A205	0.6342	0.6190	0.6524	0.5399	0.6740	0.6533
Average	0.6686	0.6910	0.6946	0.6569	0.6819	0.6864
Standard deviation	0.0918	0.0854	0.0651	0.1057	0.0662	0.0725

decrease of (H_e) could be explained by the moderate diversity in 205 locus (0.5399).

The genetic variability quantified by AMOVA showed a slight structuration between the populations of Houbara bustards, with 92.25% variation within the populations compared with only 7.75% between the populations. However, the coefficient of differentiation was not significant at 5% ($F_{ST} = 0.077$, P -value < 0.05) (Table 3).

From the indices of fixation F_{ST} presented in (Table 4), the 2006 wild population is shown to be statistically different from all other captive and founder populations.

3.2. Measurement of Genetic Diversity in Terms of Mean Coancestry

The allelic richness varies between 5 and 9 for loci D117 and A205, respectively (Figure 1). According to (Table 5), the Captive population has the highest values of inbreeding coefficient ($F_i = 0.4014$), molecular average ($f_{ii} = 0.3430$) and individual self coancestry coefficient ($s_i = 0.7007$) values, which also coincides with the decline in genetic diversity immediately after the establishment of the captive breeding program (Figure 2) and can therefore suggest the possibility of maintaining this population under a strictly closed system.

In parallel with the analysis carried out by the METAPOPOP software, the average coancestry over the entire metapopulation was calculated by MOLKIN v.3.0 and estimated at $\bar{f} = 0.313104$. As already mentioned, the captive population is issued from the crosses between individuals belonging to the founding populations of Erfoud, Errachidia and Boudnib. This can explain, on one hand, the high values of the coefficients f_{ij} (0.3354, 0.3221 and 0.3232) and on the other hand, the small values of Nei's distances among individuals corresponding to them (0.0070, 0.0042 and 0.0109) (Table 6).

The wild population in 2006 (CO6) is the most distant of the other populations (D_{ij} ranging from 0.0794 to 0.0986). The mean minimum Nei distance of the entire meta-population is estimated at ($D_{ij} = 0.0226$). This is also equal to inter-population genetic diversity $\tilde{f} - \bar{f} = [1 - \tilde{f}] - [1 - \bar{f}]$. This result is in support with the result obtained by Harlequin in Table 3, *i.e.* the significant

Table 3. Analysis of Molecular Variance (AMOVA).

Source of variation	Degree of freedom	Sum of squares	% of variation
Inter-populations	4	64.693	7.75
Intra-populations	1593	1520.577	92.25
Total	1597	1585.270	100.00

Table 4. Pairwise F_{ST} among houbara subpopulations (below the diagonal) and the corresponding P -value (above the diagonal).

	Erfoud	Boudnib	Errachidia	Captive	CO6
Erfoud	*	0.6666	0.5135	0.5945	0.0000
Boudnib	-0.0046	*	0.7297	0.2522	0.0000
Errachidia	-0.0001	-0.0046	*	0.2703	0.0000
Captive	-0.0017	0.0042	0.0043	*	0.0000
CO6	0.1349	0.1301	0.1227	0.1554	*

Table 5. Subpopulation parameters and their averages. f_{ii} : Coancestry coefficient of a subpopulation, s_i : Coefficient of self coancestry of individuals within a subpopulation, F_i : Inbreeding coefficient of a subpopulation, and d_{ii} : Distance between individuals within a subpopulation.

Subpopulation	f_{ii}	s_i	F_i	d_{ii}
Erfoud	0.3344	0.6667	0.3333	0.3223
Boudnib	0.3254	0.6429	0.2857	0.3175
Errachidia	0.3094	0.6676	0.3352	0.3582
Captive	0.3430	0.7007	0.4014	0.3577
CO6	0.3216	0.6955	0.3910	0.3739
Average	$\tilde{f} = 0.3361$	$\tilde{s} = 0.6938$	$\bar{F} = 0.3875$	$\bar{D} = 0.3576$

Table 6. Pairwise coancestry coefficient (f_{ij}) and Nei's distance among individuals ($D_{ij(Nei)}$).

Subpopulations	f_{ij}	$D_{ij(Nei)}$
Erfoud & Boudnib	0.3220	0.0105
Erfoud & Errachidia	0.3171	0.0096
Erfoud & Captive	0.3354	0.0070
Erfoud & CO6	0.2412	0.0912
Boudnib & Errachidia	0.3101	0.0073
Boudnib & Captive	0.3232	0.0109
Boudnib & CO6	0.2346	0.0886
Errachidia & Captive	0.3221	0.0042
Errachidia & CO6	0.2360	0.0794
Captive & CO6	0.2337	0.0986

value of genetic structuring measured by the index F_{ST} to 0.07752 (AMOVA).

If the different populations are forced to contribute to the next generation, the components of the overall metapopulation genetic diversity DG_T could be partitioned as mentioned in (Table 7).

With

$$DG_T = 1 - \bar{f} = [(1 - \tilde{s}) + (\tilde{s} - \tilde{f})] + [\tilde{f} - \bar{f}] = [1 - \tilde{f}] + [\tilde{f} - \bar{f}]$$

$$= 0.3062 + 0.3576 + 0.0226 = 0.6639 + 0.0226 = 0.6865$$

The population born in captivity contributed the most to total diversity with a value of 70% (0.4796). This may be due to the high number of individuals but especially to its within subpopulation genetic diversity which is equal to 0.4621. The total inferred genetic diversity is similar to that estimated by Nei's method [26] (Table 2).

Estimating the loss and gain of genetic diversity allows better management of stocks and varieties to be retained. This estimate is done by retrieving one or more populations from the gene pool and recalculating the total gene diversity (or its counterpart, i.e.: average coancestry) (Table 8). The removal of the Captive population will increase the overall genetic diversity of the meta-population

Table 7. Contribution of each subpopulation to the total gene diversity. (WI: within individual, BI: between individual, WS: within subpopulation, BS: between subpopulation, \tilde{s} : Mean self-coancestry, \tilde{f} : Mean coancestry, and \bar{f} : Mean coancestry over the entire metapopulation).

Subpopulation	Contribution to the DG_{wi}	Contribution to the DG_{bi}	Contribution to the DG_{ws}	Contribution to the DG_{bs}	Contribution to the DG_T
Erfoud	0.0104	0.0101	0.0205	0.0007	0.0213
Boudnib	0.0094	0.0083	0.0177	0.0006	0.0183
Errachidia	0.0370	0.0399	0.0769	0.0022	0.0792
Captive	0.2105	0.2516	0.4621	0.0175	0.4796
CO6	0.0389	0.0477	0.0866	0.0089	0.0955
Total	$1 - \tilde{s} = 0.3062$	$\tilde{s} - \tilde{f} = 0.3576$	$1 - \tilde{f} = 0.6639$	$\tilde{f} - \bar{f} = 0.0226$	$1 - \bar{f} = 0.6865$

Table 8. Global genetic diversity ($DG_T | i$) after the elimination of each subpopulation i and percentage of gain (+) or loss (-) of gene diversity (H_T).

Subpopulation removed	$DG_T i$	Within subpopulation (Intra-individuals)	Within subpopulation (Inter-individuals)	Among subpopulations	H_T
Erfoud	0.6870	-0.1274	0.1662	0.0331	0.07
Boudnib	0.6863	-0.2002	0.1580	0.0160	-0.02
Errachidia	0.6853	-0.4781	-0.0098	0.3141	-0.17
Captive	0.7218	2.2777	-0.0115	2.6324	4.89
CO6	0.6639	0.0381	-0.3588	-3.0796	-3.40

by 4.89%, whereas, the one of the wild CO6 population will result in a 3.40% loss in overall gene diversity due to decreased diversity or between subpopulation distance (-3.07%).

3.3. Measurement of Allelic Richness with Rarefaction

The allelic richness with K (42) or without rarefaction K in all the subpopulations studied is presented in **Table 9**.

The proportions of contribution of each population to the total allelic diversity obtained after rarefaction are summarized in (**Table 10**). The total allelic diversity is estimated to $A_T = 4.6723$ and it corresponds to the sum of the within subpopulation allelic diversity ($A_S = 4.0733$) and between subpopulation diversity ($D_A = 0.5990$). Thanks to its allelic among subpopulation distance D_A , the wild CO6 population contributes the most to the total allelic diversity. The Captive and Erfoud populations contribute the least to allelic diversity with values of 0.88 and 0.87, respectively. CO6 is the most divergent of other populations due to the number of private alleles it possesses. From the results of **Table 10**, the coefficient of allelic differentiation A_{ST} was estimated at 0.1282.

The percentages of gain (-) and loss (+) of allelic diversity recalculated after elimination of each bustard subpopulation are shown in (**Table 11**). The results obtained are in agreement with those mentioned in (**Table 10**), which show that the CO6 population is the most favorable for the program and conservation

Table 9. Number of rare alleles and allelic richness K obtained after rarefaction on a common number of 42 genes.

Subpopulation	No. of private alleles	No. of alleles/locus (K)	No. of alleles/locus after rarefaction K(42)
Efoud	0	5.00	4.88
Boudnib	0	5.25	5.25
Errachidia	0	5.75	5.19
Captive	0	6.50	4.82
CO6	11	6.50	5.23
Total	11	7.00	5.29

Table 10. Contribution (in %) of each subpopulation to the total allelic diversity of the metapopulation after rarefaction. (A_S = Allelic diversity within subpopulations, D_A = Mean distance between subpopulations, and A_T = Total allelic diversity).

Subpopulation	Within subpopulation level	Between subpopulation level	Total
Erfoud	0.7766	0.0961	0.8727
Boudnib	0.8500	0.1069	0.9569
Errachidia	0.8387	0.1064	0.9450
Captive	0.7620	0.1248	0.8868
CO6	0.8461	0.1649	1.0109
Total	$A_S = 4.0733$	$D_A = 0.5990$	$A_T = 4.6723$

priorities, especially in the long term, unlike the Captive and Erfoud populations.

3.4. Subpopulation Contribution to the Total Genetic Diversity

The optimal contributions of each population to an artificial germplasm are presented in (Table 12) by considering different values of the population factor λ . When ($\lambda = 1$), the Errachidia and wild population of 2006 will contribute the most to maximum genetic diversity ($=0.7239$) by 52.3% and 46.1%, respectively. Depending on the purpose of each conservation program, if the objective is to maximize the between subpopulation component of the f_{ij} (i.e. maximizing the distance between populations), the factor λ must be zero, so that $H_T = D_{ST}$. If the interest is to minimize total coancestry and thus maximizing total diversity, λ must take a value equal to 1. Finally, if much importance is given to the within subpopulation component, the value of λ must be greater than 1.

4. Discussion

Management of genetic variation is critical for vulnerable species raised in captivity in reserves (*ex situ*), and for wild animal species living in their original and natural habitats (*in situ*). In captive breeding systems, the regular recruitment of new wild populations is often beneficial for stable and sustainable maintenance of genetic variability. However, wild founder populations may be spatially structured and fragmented, and this differentiation in finite and isolated populations may lead to the appearance of consanguinity and genetic homogeneity, and

Table 11. Gain (-) or loss (+) of allelic diversity (in %) after retrieving each population.

Excluded subpopulation	Level (intra-population)	Level (inter-population)	Total
Erfoud	-1.0181	-0.9190	-1.9371
Boudnib	0.9455	-0.0617	0.8838
Errachidia	0.6421	-0.5114	0.1307
Captive	-1.4094	0.5196	-0.8897
CO6	0.8399	3.1249	3.9648

Table 12. Contribution of each population (in %) to a synthetic gene pool with the maximum genetic diversity (DGpool), based on different values of the population factor λ .

Subpopulation	Contribution (%)			
	$\lambda = 0$	$\lambda = 1$	$\lambda = 2$	$\lambda = 5$
Erfoud	3.10	0.00	0.00	0.00
Boudnib	3.40	1.50	0.00	0.00
Errachidia	0.00	52.3	57.7	69.3
Captive	44.1	0.00	0.00	0.00
CO6	49.1	46.1	42.3	30.70
DG _{pool}	0.7167	0.7239	0.7237	0.7201

consequently to the deterioration of overall genetic variability.

For Houbara bustards bred in captivity, the use of selectively neutral molecular markers allowed a better assessment and quantification of genetic diversity (*i.e.*: gene and allelic diversity) at both within and between subpopulation levels. Consequently, several findings have emerged and have been shown to be effective for *ex situ* conservation priorities and policies.

Calculations of genetic diversity of each subpopulation, as well as of the overall metapopulation showed that the wild populations of Errachidia, Boudnib and CO6 possess the most diversity compared to the wild population of Erfoud and Captive of 1995-2004. The average inbreeding and coancestry coefficients confirmed the origin of the Captive population from the wild populations of Erfoud-Errachidia-Boudnib area. The partitioning of total genetic diversity has also made it easy to optimize the contribution of each population to an artificial gene pool with the maximum genetic diversity. According to Eding *et al.* [27] and Fabuel *et al.* [28], the choice of the value of the population factor λ will depend on the objective and the final goal to be achieved in this optimization. Indeed, if a short-term selection response is aimed, the most frequent and common alleles should be favored, in which case the parameter λ will take small values. On the contrary, if the private alleles are to be maintained and preserved in the artificial population, the λ factor must take on larger values.

From a more general perspective of conservation and management of the Houbara breeding flock, if the ultimate objective of conservation is to maintain among subpopulation diversity, the wild population CO6 and the Captive population will be the most favored for two reasons: 1) they are the most distant ($D_{ij} = 0.986$), and 2) the proportions of contribution to a synthetic population are considerable (49.1% and 44.1% for a value of $\lambda = 0$).

However, more attention is needed during cross-breeding operations to avoid the risk of depression of exogamy. If the conservation strategy and priorities are to preserve intra-population diversity, the Errachidia population and the CO6 wild population should be favored given their large contribution proportions for $\lambda = 2$ and $\lambda = 5$.

In practice, the most widely adopted approach for maintaining genetic diversity, restricting and limiting inbreeding depression is to optimize parental contributions to the next generation through minimization of the overall coancestry of a particular metapopulation [29]. This strategy leads to a maximization of the global genetic diversity in terms of expected heterozygosity and effective population size [30].

In conclusion, the partition of allelic richness proposed by Petit *et al.* [5] is dependent only on the number of private alleles present in a population, so that the population can contribute to total allelic richness only if it has rare and unique alleles, otherwise its contribution will be zero. In contrast to El Mousadik and Petit [22] and Petit *et al.* [5], the procedure proposed by Caballero and Rodríguez-Ramilo [23] takes into account rare alleles and common alleles in the estimation of between subpopulation allelic differences.

From a long-term perspective, allelic richness is more advantageous than gene diversity for two reasons. First, it is the most sensitive to bottleneck events and therefore better reflects the old fluctuations of the effective population size [31]. Second, the limit of response to selection is often determined by the initial number alleles in a population [32]. However, short-term responses to inbreeding selection and depression are directly related to gene diversity.

In fact, in the selection of parents, the system of captive crosses must be added, since structuring and genetic differentiation in a meta-population is directly related to the type of coupling regime applied (circular, rotational, etc.) [33]. However, the impact of genetic diversity on maximizing genetic diversity is generally less important than the contribution of parents.

At the end of this comparative study, allele and gene diversities are two important criteria, which are not necessarily equivalent but are complementary, especially when it comes to preserving genetic diversity and identification of conservation units.

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