Genetic assessment of a conservation breeding program of the houbara bustard (*Chlamydotis undulata undulata*) in Morocco, based on pedigree and molecular analyses

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Robin Rabier, Alexandre Robert, Frédéric Lacroix, Loïc Lesobre. Genetic assessment of a conservation breeding program of the houbara bustard (*Chlamydotis undulata undulata*) in Morocco, based on pedigree and molecular analyses. Zoo Biology, 2020. hal-03020087

HAL Id: hal-03020087

<https://hal.sorbonne-universite.fr/hal-03020087>

Submitted on 23 Nov 2020

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1 Rabier

Genetic assessment of a conservation breeding program of the Houbara bustard (*Chlamydotis undulata undulata*) in Morocco, based on pedigree and molecular analyzes.

Genetics of conservation breeding program

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ABSTRACT

Protection and restoration of species in the wild may require conservation breeding programs under genetic management to minimize deleterious effects of genetic changes that occur in captivity, while preserving populations' genetic diversity and evolutionary resilience. Here, through interannual pedigree analyzes, we first assessed the efficiency of a 21-year genetic management, including minimization of mean kinship, inbreeding avoidance, and regular addition of founders, of a conservation breeding program targeting on Houbara bustard (*Chlamydotis undulata undulata*) in Morocco. Secondly, we compared pedigree analyzes, the classical way of assessing and managing genetic diversity in captivity, to molecular analyzes based on seven microsatellites. Pedigree-based results indicated an efficient maintenance of the genetic diversity (99% of the initial genetic diversity retained) while molecular-based results indicated an increase in allelic richness and an increase in unbiased expected heterozygosity across time. The pedigree-based average inbreeding coefficient F remained low (between 0.0004 and 0.003 in 2017) while the proportion of highly inbred individuals ($F > 0.1$) decreased over time and reached 0.2% in 2017. Furthermore, pedigree-based F and molecular-based individual multilocus heterozygosity were weakly negatively correlated, (Pearson's

2 Rabier

$r = -0.062$ when considering all genotyped individuals), suggesting that they cannot be considered as alternatives, but rather as complementary sources of information. These findings suggest that a strict genetic monitoring and management, based on both pedigree and molecular tools can help mitigate genetic changes and allow to preserve genetic diversity and evolutionary resilience in conservation breeding programs.

KEYWORDS

Ex situ, inbreeding avoidance, mean kinship, heterozygosity, genetic management

INTRODUCTION

In critical situations such as highly degraded environment or significant threats to wild populations, conservation breeding programs are essential to support *in situ* conservation measures and ensure the persistence of endangered species (Conde, Flesness, Colchero, Jones, & Scheuerlein, 2011; IUCN, 2014; Pritchard, Fa, Oldfield, & Harrop, 2012). Indeed, from a conservation perspective, the combined use of both *in* and *ex situ* approaches is recognized as a more effective strategy than using either one of them (Pritchard et al., 2012; Redford, Jensen, & Breheny, 2012; Volis & Blecher, 2010). However, captive breeding can be associated with genetic changes that might affect present and future eco-evolutionary trajectories of populations. Most expected changes are a reduction of genetic diversity through genetic drift (Lacy, 1987), inbreeding and associated inbreeding depression (Hedrick & Kalinowski, 2000; Keller & Waller, 2002), adaptation to captivity (Christie, Marine, French, & Blouin, 2012; Frankham, 2008), and the drift load associated to relaxed selection in small populations (Robert, 2009). Concerning translocations from captive to wild populations, both empirical (Araki, Cooper, & Blouin, 2007) and theoretical (Robert, 2009) studies indicated that the time a population spent in captivity can be strongly and negatively correlated to the fitness of the population in the wild, therefore jeopardizing the success of the conservation program (Lynch & O'Hely, 2001). Consequently, strict genetic management is required in order to mitigate deleterious effects of these potential genetic changes during the captive phase of conservation programs (IUCN, 2014). While providing individuals for wild supplementation, conservation breeding programs must maintain their genetic diversity at a threshold that preserves populations' evolutionary resilience, *i.e.*

3 Rabier

21 the ability of populations to persist in their current state and undergo evolutionary adaptation in

22 response to changing environmental conditions (Sgrò, Lowe, & Hoffmann, 2011). Released

23 individuals must exhibit enough genetic diversity in order not to alter the gene pool of wild

24 populations (Kleiman, Price, & Beck, 1994). Thereby, management strategies must address the trade-

25 off between genetic goals (*i.e.* maintenance of genetic diversity) and demographic goals (*i.e.* provision

26 of a sufficient number of individuals to provide significant support for *in situ* conservation actions)

27 (Ballou, 1992; Ballou et al., 2010; Lacy, 1994). Some authors advocated the maintenance of 90% of

28 the initial genetic diversity after 200 years in captivity (Soulé, Gilpin, Conway, & Foose, 1986), while

29 others, focused on inbreeding, recommending to keep the individual inbreeding coefficient below 0.1,

30 a level above which inbreeding depression can significantly affect individuals' fitness (Huisman,

31 Kruuk, Ellis, Clutton-Brock, & Pemberton, 2016; Ralls et al., 2018). Furthermore, minimization of

32 adaptation to captivity can be achieved through reduction of the number of generations spent in

33 captivity, equalization of family size (Allendorf, 1993; Williams & Hoffman, 2009), or through the

34 maintenance of a gene flow between wild and captive populations (Conway, 1995).

35 Usually, estimating and managing genetic diversity in captive populations is achieved using pedigree

36 analyzes (Frantzen, Ferguson, & de Villiers, 2001; Hedrick & Fredrickson, 2008; Lacy, Ballou,

37 Princee, Starfield, & Thompson, 1995; Nagy et al., 2010). However, the development of molecular

38 tools such as microsatellites or, more recently, genomic markers has paved the way for new ways of

39 assessing genetic diversity (Allendorf, Hohenlohe, & Luikart, 2010). Molecular- and pedigree-based

40 metrics cannot be considered as alternatives but rather as complementary sources of information since

41 they rely on distinct types of information collected at different biological scales (*i.e.* reproduction

42 events within a population *vs.* variation of DNA), they are not based on equivalent assumptions, and

43 might suffer different technical and statistical limitations (Nietlisbach et al., 2017; Ruiz-López,

44 Roldán, Espeso, & Gomendio, 2009; Slate et al., 2004; Wang, 2016; Witzemberger & Hochkirch,

45 2011). One of the strongest assumptions underlying pedigree analyzes is that founders are neither

46 inbred nor related, and that the variance in relatedness amongst them is null (hereafter “founders

47 assumption”; Hogg et al., 2019; Ruiz-López et al., 2009). This assumption is subject to caution when

4 Rabier

founders are collected in wild populations that may already suffered from the genetic consequences of a demographic bottleneck (Hammerly, Morrow, & Johnson, 2013; Ruiz-López et al., 2009) or when captive and wild populations are highly related. The potential divergence between molecular and pedigree approaches is illustrated by the low consistency of pedigree-based inbreeding coefficient and molecular-based heterozygosity reported in the literature (see for example Slate et al., 2004). In this context, it seems particularly important to combine these two approaches in order to better understand their divergences, complementarity, and usefulness for genetic management.

Within this framework, we assessed genetic diversity levels and consistency between pedigree and molecular analyzes in a large captive population of the threatened North-African Houbara bustard (*Chlamydotis undulata undulata* Jacquin 1784, hereafter Houbara). The Houbara is a promiscuous bird with an “exploded-lek” mating system (Hingrat et al., 2004), historically distributed from North Mauritania to Egypt. As a consequence of unregulated hunting, poaching, and habitat degradation (Azafzaf, Sande, Evans, & Collar, 2005; Goriup, 1997), the species has suffered a sharp population decline since the 1990s with an estimated population decline of 25% between 1984 and 2004 (BirdLife International, 2018). The Houbara is listed under the Appendix 1 of the Convention on International Trade of Endangered Species (CITES, <https://cites.org/eng/node/20646>) and classified as “Vulnerable” in the Red List of the International Union for Conservation of Nature (IUCN, 2016). In 1996, this decline led to the establishment of a conservation breeding program in Morocco: the Emirates Center for Wildlife Propagation (ECWP, www.ecwp.org), a project of the International Fund for Houbara Conservation (IFHC, www.houbarafund.org), aiming to restore sustainable free-ranging populations of Houbara (Lacroix, Seabury, Al Bowardi, & Renaud, 2003). This program is managed as a captive-free-ranging system with regular exchanges between captive and free-ranging populations through supplementation of wild populations with captive bred individuals along with regular additions of founders using egg collections in the wild. In addition, and in order to maximize its genetic diversity, ECWP’s captive population is under a strict genetic management primarily based on a strategy of minimizing mean kinship (see Methods) through pedigree analyzes (Lesobre, 2008).

1 5 Rabier

2
3 74 Thanks to this large 21-year complete pedigree and to the existence of a microsatellite genotyping
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5 75 dataset, we used ECWP’s captive population of Houbara as a model to evaluate the combined use of
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7 76 pedigree and molecular analyzes in monitoring the maintenance of genetic diversity in conservation
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9 77 breeding programs.

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12 78 **METHODS**

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15 79 Populations studied and conservation project

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17 80 Houbara populations, spreading from the Atlantic coast of Morocco to the Sinai desert in Egypt, are
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19 81 lacking genetic differentiation and are, therefore, managed as a single conservation unit (Lesobre,
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21 82 Lacroix, Caizergues, et al., 2010). In order to restore self-sufficient Houbara populations throughout
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23 83 its range, ECWP’s conservation strategy combines both *in situ* (e.g. ecological studies, hunting
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25 84 regulation and management, socioeconomic development) and *ex situ* conservation actions (*i.e.*
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27 85 preservation of the species’ genetic diversity in captivity and provision of surplus birds to
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29 86 complement *in situ* conservation actions). The captive population was created in 1996, with 52
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31 87 founders, collected in Algeria in 1986 and 1987, and their 244 descendants, transferred from the
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33 88 National Wildlife Research Center (Taïf, Saudi Arabia) (Lesobre, 2008). Subsequently, founders were
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35 89 added to the captive population during three major egg collections within free-ranging populations of
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37 90 eastern Morocco, *i.e.* 115 eggs between 1996 and 2001, 479 between 2002 and 2009, and 191 eggs
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39 91 between 2015 and 2017. Wild eggs’ nest of origin was systematically recorded (as well as the female
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41 92 identity when the female has been previously tagged) and, in order to adopt the more conservative
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43 93 strategy, eggs collected from a single nest were considered as having the same wild father, *i.e.* they
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45 94 were considered as full sibs, during pedigree analyzes. Between 1997 and 2017, 198 556 chicks
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47 95 hatched in captivity, of which 133 423 were released into the wild.

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51 96 Conservation breeding management

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54 97 Within ECWP, birds are housed individually, and reproduction is performed artificially, through
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56 98 sperm collection, insemination, and incubation. This ensures pedigree accuracy, thus allowing strict
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58 99 genetic management through pedigree analyzes. The genetic management strategy implemented in
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6 Rabier

2002 (Lesobre, 2008), *i.e.* pairing management and identification of surplus individuals, is based on (i) minimization of mean kinship within the captive population, (ii) avoidance of inbreeding, and (iii) equalization of family size to prevent risk of adaptation to captivity (Allendorf, 1993). Both simulation (Ballou & Lacy, 1995; Giglio et al., 2016) and empirical (Montgomery et al., 1997; Willoughby et al., 2017) studies pointed out that minimizing mean kinship by preferentially breed individuals descended from underrepresented founders (*i.e.* individuals with rare genotype relative to the whole population) is an effective strategy in maximizing genome-wide variation, gene diversity, and allelic diversity. Thus, it allows taking into account factors that impact genetic composition of the captive population (*e.g.* mortality, reproductive success) (Ballou & Lacy, 1995; Rudnick & Lacy, 2008). The identification of surplus individuals for supplementation releases is based on the *Genetic Dumping Strategy*, *i.e.* optimization of the captive population's genetic diversity, and equalization of family size (Earnhardt, 1999). According to these principles, each chick is assigned either for the renewal of the captive population or for the reinforcement of the free-ranging population and will thus follow specific rearing protocols. Hereafter, these groups are referred as *breeding chicks* and *surplus chicks* respectively while the *breeding flock* is composed of all captive bred adults within the captive population.

116 Microsatellite genotyping

DNA extractions were performed using NucleoSpin-tissue kits, created by Macherey-Nagel (Düren, Germany). The genotyping of 7 microsatellite loci (A113a, A120, A2, A21, A210, A29, D118), designed for *C. u. undulata* (Chbel, Broderick, Idaghdour, Korrida, & McCormick, 2002) was made by GENOSCREEN (Lille, France). Due to the presence of null allele, the locus A113a was amplified with new primers. The primers used were 5'-GTTGTGTGTCCTGGGAGCAGC-3' and 5'-TGGTGAGCTTTCTTCAA-3'. Amplifications were performed by multiplex PCR in a final volume of 20 µl, containing 0.2 µl of Taq Polymerase (5 U/ml), 1.5 µl of dNTPs (0.24 mM), 1.5 µl of MgCl₂ (1.5 mM), and 2 µl of DNA. The primer concentration for each locus ranged from 0.125 µM to 1 µM. PCR conditions were: hot start at 94°C for 10 min, followed by 40 cycles at 94°C for 30 sec, 55°C for 1 min, and 72°C for 30 sec, with a final extension step at 72°C for 10 min. Amplification products

7 Rabier

127 were analyzed by an automatic sequencer (3730XL DNA analyzer, Applied Biosystem) and allele
128 sizes were assessed by GENMAPPER software.

129 Pedigree analyzes

130 During a single breeding season, females can be inseminated with sperm of various males; combined
131 with the occurrence of sperm retention and competition in the species, this led to the existence of
132 dubious paternities within the captive population (Lesobre, 2008). In order to improve pedigrees
133 accuracy, paternity analyzes, based on mendelian inheritance, were conducted using CERVUS 3.0.7
134 (Kalinowski, Taper, & Marshall, 2007) and the seven microsatellites markers. Every dubious
135 paternity within the captive population were resolved through molecular data; between 1997 and
136 2017, this represented 33% of the breeding chicks. Pedigree analyzes were conducted annually, from
137 1997 to 2018 for the breeding flock (range of pedigree sizes [231, 8 631]) and from 1997 to 2017 for
138 both breeding (range [165, 659]) and surplus chicks (range [13, 21 545]). The package *optiSel* 2.0.2
139 (Wellmann, 2018), in R 3.5.1 (R Core Team, 2018) was used to perform pedigree analyzes and to
140 compute pedigree's proportion of known ancestry, individual generation, and the kinship between
141 individuals as the probability of alleles to be identical by descent. Kinship results were used to
142 compute individual mean kinship M_k as the average kinship of an individual with the whole
143 population (Ballou & Lacy, 1995; Lacy, 1995), and the individual inbreeding coefficient (F) as the
144 kinship of the parents of the focal individual (Keller & Waller, 2002). The population average
145 inbreeding coefficient by year is noted $\bar{F}_{year}$. During pedigree analyzes, unknown parents were
146 considered as individuals of generation 0, not related to the captive population (*i.e.* $M_k = 0$), of zero
147 inbreeding. These individuals were not considered as founders when computing the number of
148 founders within the pedigree. Individuals with two wild parents were considered as founders and wild
149 bred individuals collected from the same nest, or from the same tagged female over different years,
150 were considered as full sibs. Founders were excluded from kinship computation. Pedigrees were well-
151 known (proportion of known ancestry $> 98\%$) and exhibited an average depth ranging from 1.03 to
152 3.68 generations in captivity. Pedigree descriptions, including pedigree sizes, proportion of known
153 ancestry, average generation, and maximum generation are provided in TABLE 1.

8 Rabier

Molecular analyzes

Breeding flocks were analyzed for the period 1997-2018 (on average 59.4% of annual breeders were genotyped; TABLE 1), these flocks were composed of captive bred individuals only. FSTAT 2.9.3.2 (Goudet, 1995) was used to compute allelic richness Ar and GENALEX 6.503 (Peakall & Smouse, 2006) was used to compute observed heterozygosity Ho , expected heterozygosity He , and unbiased expected heterozygosity uHe , across the 7 microsatellites. Individual multilocus heterozygosity (MLH) was calculated as the proportion of typed loci at which an individual was heterozygous (Coltman, Pilkington, Smith, & Pemberton, 1999).

Comparison of molecular- and pedigree-based estimates of inbreeding

In order to compare pedigree- and molecular-based metrics related to inbreeding, we used the pedigree-based individual inbreeding coefficient F and the molecular-based individual multilocus heterozygosity MLH (Coltman & Slate, 2003; Ruiz-López et al., 2009; Slate et al., 2004). A negative correlation was expected between F and MLH since inbred individuals are likely to have similar alleles at homologous sites (Lacy, 1995; Whitlock, 2004). Only individuals with 7 typed loci were considered ($N = 7\ 158$). Founders were not included in the analysis to avoid bias since their individual inbreeding coefficient was set to zero because of the “founders assumption” (Ruiz-López et al., 2009). Different batches were made, *i.e.* by sex, by range of individual inbreeding coefficient, and by generation in order to test the effect of pedigree depth on the F /MLH correlation (Nietlisbach et al., 2017; Slate & Pemberton, 2002).

Statistical analyzes

All statistics were conducted in R 3.5.1 (R Core Team, 2018). Conformity to the Hardy-Weinberg equilibrium was tested using the package *genepop* 1.0.5 (Rousset, 2008). P-values were calculated using Markov chains (dememorization = 10 000; batches = 20; iterations per batches = 5 000). Interannual variations of Ar , Ho , and uHe were assessed using mixed effects linear models (package *nlme* 3.1.137; Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2018) with the year as a fixed effect variable and the locus as a random effect variable. Correlation between heterozygosities at each locus

9 Rabier

180 (hereafter heterozygosity-heterozygosity correlation) was tested using the package *Rhh* 1.0.1 (Alho,
181 Välimäki, & Merilä, 2010). It was calculated by repeatedly and randomly dividing typed loci in two
182 sets, and calculating an estimate of individual MLH for both sets of loci (Alho et al., 2010). One
183 hundred thousand iterations were used. Interannual variations of average mean kinship were tested
184 with mixed effects linear models (package *nlme* 3.1.137; Pinheiro et al., 2018) with the year, the
185 group (*i.e.* breeding flock, breeding chicks, or released chicks), and their interaction as fixed effect
186 variables and the individual identity as a random effect variable. Because of the large number of
187 individuals exhibiting a null individual inbreeding coefficient (between 24% of released chicks in
188 2007 and 100% of the breeding flock in 1997; Fig. 1B), inbreeding was also analyzed in terms of
189 proportions of individuals exhibiting an individual inbreeding coefficient higher than 0.1 (Ralls et al.,
190 2018). Interannual variations of proportions of highly inbred individuals ($F > 0.1$) were assessed using
191 generalized linear models with binomial distribution. The analyzed variable was a binomial variable
192 equal to 1 if the individual exhibited an individual inbreeding coefficient equal to or above 0.1 and
193 equal to 0 otherwise. The year was considered as a quantitative variable. R^2_{fix} corresponds to the
194 coefficient of determination computed for the fixed effects of the model (package *MuMIn* 1.42.1;
195 Barton, 2018).

196 Since F and MLH data were not normally distributed and since pedigree-based F resulted in an excess
197 of ties, the F /MLH correlation was tested using, both, a parametric Pearson's test, and a non-
198 parametric Kendall's rank test. These two approaches provided similar results when including all
199 genotyped individuals (Pearson's $r = -0.061$ and Kendall's tau = -0.062). Thus, we retained Pearson's
200 test (which is more appropriate in the case of ties excess) to make subsequent comparison of various
201 batches (*i.e.* by sex, by range of individual inbreeding coefficient, and by generation). In these tests,
202 p-values were adjusted for multiple comparisons using Bonferroni correction (Rice, 1989). Note that
203 confirmation of paternities using molecular data did not bias our results since (i) in paternity analyzes,
204 the information extracted from molecular data is relative to a vertical similarity (*i.e.* father-offspring),
205 while for the computation of genetic diversity indices, the information is relative to a horizontal
206 similarity; (ii) if it skewed our results, it would be toward a strong F /MLH correlation (in absolute

10 Rabier

value), which was not the case (see Results); and (iii) the F/MLH correlation when considering only individuals without paternity confirmed using molecular data was as weak as that calculated for all genotyped individuals.

RESULTS

Pedigree analyzes

The average mean kinship exhibited low levels in 1997 (0.030 ± 0.015 for the breeding flock, 0.034 ± 0.014 for breeding chicks, and 0.054 ± 0.016 for surplus chicks) and further decreased over time (p-value < 0.001 for the three groups, $R^2_{\text{fix}} = 0.59$; Fig. 1A). Note that the average mean kinship of surplus chicks had converged to the values of the captive groups (Fig. 1A). The average inbreeding coefficient $\bar{F}_{\text{year}}$ increased for the breeding flock and for breeding chicks during the growth phase of the program and prior to the implementation of the current genetic management in 2002. However, it remained low since 1997 ($\bar{F}_{\text{year}} \leq 0.011$ for the breeding flock, $\bar{F}_{\text{year}} \leq 0.016$ for breeding chicks, and $\bar{F}_{\text{year}} \leq 0.073$ for surplus chicks; Fig. 1B). In the same way, the proportion of individuals with relatively high individual inbreeding coefficient ($F > 0.1$) decreased with time, for the breeding flock (p-value < 0.001), for breeding chicks (p-value < 0.001), and for surplus chicks (p-value < 0.001) (Fig. 1C). Proportions of inbred individuals in the captive population in 2017 are provided in TABLE 2. Note that in 1997, the average inbreeding coefficient and the average mean kinship were different from zero since the captive population was composed of both wild and captive bred birds (*i.e.* 74 founders and 231 captive bred birds descended from 49 founders; TABLE 1).

Molecular analyzes

Loci A113a, A210, A29, and A2 exhibited discrepancies from the Hardy-Weinberg equilibrium (TABLE 3). The average allelic richness of the breeding flock increased from 5.84 ± 2.74 in 1997 to 7.39 ± 4.00 in 2018 (p-value < 0.001; Fig. 2A). The unbiased expected heterozygosity of the breeding flock also increased (p-value < 0.001; Fig. 2B) from 0.66 ± 0.16 in 1997 to 0.70 ± 0.14 in 2018; while the observed heterozygosity of the breeding flock did not show significant evolution over time (p-value = 0.963; Fig. 2B). It was equal to 0.67 ± 0.19 in 1997 and 0.69 ± 0.13 in 2018. The

11 Rabier

heterozygosity-heterozygosity correlation between loci was weak ($r = 0.011$; 95% IC: -0.004 to 0.026) when considering all individuals that have been genotyped.

Comparison of pedigree-based inbreeding coefficient F and multilocus heterozygosity MLH

The correlation between pedigree-based individual inbreeding coefficient F and molecular-based individual multilocus heterozygosity MLH was usually negative, as expected, but remained weak (TABLE 4; Fig. 3A). When considering all individuals that have been genotyped, correlation coefficients were low and negative for both parametric and rank-based tests, *i.e.* -0.061 (p-value < 0.001) for Pearson's test (TABLE 4) and -0.062 (p-value < 0.001) for Kendall's test. However, the correlation tended to increase, in absolute value, with the number of generations and was slightly more important for males than for females (respectively Pearson's $r = -0.074$ and Pearson's $r = -0.062$; TABLE 4). Conversely, no trend was identified when comparing batches by level of individual inbreeding coefficient (TABLE 4). Note that, the average inbreeding coefficient of the group of genotyped individuals ($N = 7\,158$) was equal to 0.006 ± 0.023 (variance equal to 0.0006) and that most individuals exhibited a null individual inbreeding coefficient (76.6% of the 7 158 genotyped individuals; Fig. 3B).

DISCUSSION

Preservation of the genetic diversity of the Houbara captive population

In the context of conservation breeding programs, previous works suggested that the supplementation of captive populations through the regular addition of individuals from the free-ranging population allows preserving the evolutionary resilience of captive-free-ranging systems through (i) maintaining the captive population's genetic diversity (Sato, Ogden, Komatsu, Maeda, & Inoue-Murayama, 2017), (ii) maintaining more genetic diversity than in isolated populations (Lacy, 1987; Margan et al., 1998), (iii) mitigating adaptation to captivity (Conway, 1995), and (iv) reducing the risk of outbreeding depression (Edmands, 2007; Weeks et al., 2011). In addition, prevalent recommendations were made for the retention of 90% of the initial genetic diversity after 200 years in captivity (Soulé et al., 1986)

12 Rabier

and the avoidance of individual inbreeding coefficient exceeding 0.1 (Ralls et al., 2018), since inbreeding can increase extinction probability in wild populations (Saccheri et al., 1998).

Thanks to the current genetic management of ECWP's captive population of Houbara, values of average mean kinship achieved since the beginning of the program showed a preservation of more than 93% of the initial genetic diversity (average M_k between 0.070 and 0.007; Fig. 1A). Similarly, average inbreeding coefficients remained weak and much lower than 0.1 (Fig. 1B), while the proportion of individuals with an individual inbreeding coefficient equal to or above 0.1 decreased (Fig. 1C). In 2017, average inbreeding coefficient ($\bar{F}_{2017}$ ranging from 0.0004 to 0.003; TABLE 2) and average mean kinship (equal to 0.008; TABLE 2) were generally lower than those of other, usually seen as, large conservation breeding programs, such as the golden-headed lion tamarins *Leontopithecus chrysomelas* (average M_k = 0.0157; Ballou & Mace, 1990), the whooping crane *Grus Americana* (average M_k = 0.0325, average F = 0.0743; Boardman, Mace, Peregoy, & Ivy, 2017), the snow leopard *Uncia Uncia* (average M_k = 0.03, average F = 0.03; Blomqvist, 2007), or the cheetah *Acinonyx jubatus* (average M_k = 0.0273, average F = 0.0024; Crosier, Moloney, & Andrews, 2017).

However, the 2017 captive population of Houbara differed from these programs because of its size (*i.e.* 8 648 individuals for Houbara *vs.* 297 for the golden-headed lion tamarins, 201 for the whooping crane, 445 for the snow leopard, and 315 for the cheetah), its number of founders (*i.e.* 262 for Houbara *vs.* 83 for the golden-headed lion tamarin, 65 for the whooping crane, 56 for the snow leopard, and 93 for the cheetah), and its strong connection to the free-ranging population through regular collection of founders. Importantly, within ECWP's captive population of Houbara, the convergence of average mean kinship values of surplus birds toward values of the breeding flock (Fig. 1A) indicated that the genetic diversity preserved in the captive population was also efficiently transferred to individuals provided for supplementation of the free-ranging population. This suggests that the current genetic management allows preserving the evolutionary resilience in both captive and free-ranging populations. However, findings were more contrasted concerning microsatellites analyzes of the breeding flock, which showed an increase in the average allelic richness and in the unbiased expected heterozygosity but no significant evolution of the observed heterozygosity (Fig. 2).

13 Rabier

285 Loci A113a, A210, A29, and A2, deviated from Hardy-Weinberg equilibrium (TABLE 3); that is
286 expected in conservation breeding programs where mating is not performed randomly and where the
287 selection of offspring is oriented thanks to genetic criteria (Gómez-Romano, Villanueva, Rodríguez
288 de Cara, & Fernández, 2013; Wang, 1996).

289 Comparison of pedigree-based inbreeding coefficient and molecular-based MLH

290 Usually, consistency between pedigree- and molecular-based measures of genetic diversity is
291 investigated through the correlation between pedigree-based individual inbreeding coefficient F and
292 molecular heterozygosity (Balloux, Amos, & Coulson, 2004; Nietlisbach et al., 2017; Ruiz-López et
293 al., 2009; Slate et al., 2004; Wells, Cant, Nichols, & Hoffman, 2018). As predicted by Balloux *et al.*
294 (2004) and Slate *et al.* (2004), the F/MLH correlation within ECWP's captive population of Houbara
295 was generally negative but remained weak (Pearson's $r = -0.061$ and Kendall's $\tau = -0.062$ when
296 considering all genotyped individuals; TABLE 4). Although this correlation tended to increase with
297 pedigree's depth, as expected (TABLE 4; Nietlisbach et al., 2017). Several hypotheses can explain the
298 relative discrepancies between pedigree- and molecular-based metrics.

299 First, these two approaches rely on different concepts. Pedigree-based individual inbreeding
300 coefficient is a measure based on probabilities of identity by descent within a genealogy, depending
301 on "founders assumption", and conveys the theoretical accumulation of inbreeding (Hogg et al., 2019;
302 Lacy, 1995; Marsden, Verberkmoes, Thomas, Wayne, & Mable, 2013; Wang, 2016). Accordingly, it
303 only reflects information contained within pedigrees (Keller & Waller, 2002), while microsatellite
304 data provide an empirical assessment of genetic variation independent of assumptions underlying
305 pedigrees and that captures natural variation amongst siblings (Wang, 2016). In particular, deviations
306 from the "founders assumption", assuming that founders are neither related nor inbred, is a major
307 explanation of the discrepancies between pedigree and molecular analyzes (Goncalves da Silva,
308 Lalonde, Quse, Shoemaker, & Russello, 2010; Ruiz-López et al., 2009). Indeed, founders of a
309 conservation breeding program are often collected within a declining and small wild population and it
310 is likely that such a population already suffered from close related mating (Brock & White, 1992;
311 Hammerly et al., 2013). Moreover, although useful for conservation purposes (see above), the

14 Rabier

312 maintenance of a gene flow between captive and free-ranging populations, through supplementation
 313 releases and regular addition of founders, can increase the relatedness between founders and the
 314 captive population. Finally, the correlation also depends on the founding population's genetic
 315 diversity (Groombridge, Raisin, Bristol, & Richardson, 2012). In fact, estimates of molecular genetic
 316 diversity depend on both the genetic diversity of founders and the genetic relationships of the
 317 individuals of the captive population. In contrast, pedigree-based ones are independent of the true
 318 level of the initial genetic diversity and only rely on pedigree structure (Ito, Ogden, Langenhorst, &
 319 Inoue-Murayama, 2017).

320 In addition, technical parameters can impact pedigree- and molecular-based indicators and their
 321 correlation. Firstly, because pedigree analyzes only reflect the information contained within the
 322 pedigree, they are widely dependent on pedigree's quality, completeness, and depth (Cortés, Eusebi,
 323 Dunner, Sevane, & Cañón, 2019; Witzenberger & Hochkirch, 2011). A high proportion of parentage
 324 mistakes or unknown individuals, assumed to be founders, may lead to wrong estimates of inbreeding,
 325 inducing a weak correlation with molecular-based indices (Hammerly et al., 2013; Slate et al., 2004).

326 Secondly, it has been shown that correlation between pedigree-based F and molecular-based metrics
 327 is weak when the individual inbreeding coefficients and their variances are low (Balloux et al., 2004;
 328 Hammerly et al., 2013; Ruiz-López et al., 2009; Slate et al., 2004). Finally, it appears that the use of a
 329 small number of microsatellite loci, exhibiting weakly correlated heterozygosities, tends to weaken
 330 the correlation (Balloux et al., 2004; Slate et al., 2004). While numerous studies indicated that few
 331 molecular markers (*i.e.* typically a dozen or less) are inappropriate to accurately estimate inbreeding
 332 and relatedness in endangered species (Blouin, 2003; Taylor, 2015; Taylor, Kardos, Ramstad, &
 333 Allendorf, 2015; Wang, 2016), there is no consensus on the number of microsatellite loci needed to
 334 compute an accurate estimate of heterozygosity, and some studies used numbers as large as 160
 335 microsatellite loci without consistency between heterozygosity and pedigree-based individual
 336 inbreeding coefficient (Nietlisbach et al., 2017). In this context, the development and the use of
 337 genomic methods (*e.g.* SNPs) should provide access to a larger number of markers and a more
 338 reliable estimate of genome-wide heterozygosity through "Runs of Homozygosity" (Allendorf et al.,

15 Rabier

2010; Attard, Beheregaray, & Möller, 2018; Galla et al., 2019; Hoffman et al., 2014; Ivy, Putnam, Navarro, Gurr, & Ryder, 2016 Wang, 2016).

Within the ECWP, the “founders assumption” is likely not entirely accurate. However, the impact of this bias is likely reduced since most founders represented in the captive population result from egg collections performed within a large area (*i.e.* the Moroccan oriental, *c.a.* 50 000 km²), with low levels of relatedness between individuals (*i.e.* a previous study showed that the average relatedness between males at a displaying sites was of 0.026; Lesobre, 2008) and before the reinforcement of the free-ranging population became significant in 2009 (see the group Surplus chicks in TABLE 1). In addition, the nest of origin and mother identity were systematically recorded for all eggs collected in the wild, allowing for the integration of their possible sibling relationships within the pedigree analyzes (*i.e.* eggs collected from a single nest were considered as full sibs). Nevertheless, future egg collections will provide individuals that are, at least partially, descendants from released individuals and further studies are ongoing to evaluate how to best include them within the conservation breeding program. Concerning pedigree’s quality, it is assured by a high proportion of known ancestry (more than 96% of known individuals; TABLE 1) and by an accuracy strengthened by additional validation of dubious paternities through microsatellites markers. Finally, according to references cited above, it is likely that the low F/MLH correlation within the ECWP is partly explained, on the first hand, by the low pedigree-based individual inbreeding coefficient as well as its variance (average F of 0.006 ± 0.023 with a variance of 0.0006), and, on the other hand, by the low number of molecular loci used and their weak heterozygosity-heterozygosity correlation ($r = 0.011$; 95% IC: -0.004 to 0.026). This suggests that this set of microsatellite markers does not provide an accurate estimate of genome-wide heterozygosity (Slate et al., 2004).

CONCLUSION

For several decades, many authors have stressed the importance of captive breeding programs in the conservation of endangered species, and have established broad principles to be followed in order to (i) capture and conserve the maximum amount of genetic diversity, (ii) minimize adaptation to captivity, and (iii) avoid excessively high levels of inbreeding. The Houbara conservation breeding

16 Rabier

366 program is part of this strict genetic management approach, while having several particularities: (i) a
 367 large number of founders from the beginning of the program; (ii) the combined use of pedigrees and
 368 molecular data to manage and monitor the genetic diversity of the captive population, with 15 393
 369 captive bred individuals monitored over 21 years; (iii) a considerable number of individuals produced
 370 and released into the wild (133 423 birds released between 1997 and 2017) to reinforce the free-
 371 ranging population; and (iv) regular exchanges between free-ranging and captive populations (*i.e.*
 372 regular addition of individuals from free-ranging population). Based on our findings and the specifics
 373 of the Houbara conservation program, we make the following five recommendations to promote
 374 compliance with the general principles of conservation genetics in any conservation breeding
 375 program.

376 First, we advocate initiating a conservation breeding program as soon as the species decline is
 377 identified to allow (i) providing suitable time to acquire the zootechnical knowledge to successfully
 378 propagate the species in captivity and (ii) collecting a sufficient number of founders without affecting
 379 wild populations.

380 Second, we recommend ensuring clear and complete pedigrees as these data can only be partially
 381 recovered through molecular analyzes. Obtaining reliable genetic estimates (*e.g.* kinship) through
 382 molecular analyzes requires a large amount of information, likely to be achieved only through the use
 383 of genomics, which would be done at a prohibitive cost for many programs (Attard et al., 2018; Ivy et
 384 al., 2016).

385 Third, we recognize that molecular data are critical to clarify pedigrees through parentage studies (*i.e.*
 386 paternity or maternity analyzes) or to obtain some degree of information about the founders'
 387 relatedness. It is then crucial to implement a bank of genome samples as early as possible, in order to
 388 maintain a complete and reliable knowledge about the genetic state of the population. For this type of
 389 analyzes, low-cost markers such as microsatellites can be used successfully. In addition, our results
 390 highlighted the complementarity between pedigree and molecular approaches. Thus, their
 391 confrontation (and potential discrepancies) represents an important source of information.

17 Rabier

Fourth, we advise to add individuals from the free-ranging population to the captive one on a regular basis to minimize genetic drift if the state of free-ranging populations allows it. These additional collections reinforce the need to use molecular data to assess the inbreeding and relatedness of founders. Thus, incorporating a founder kinship matrix into pedigree analyzes is an important perspective of the Houbara conservation program.

Finally, it is crucial to have a thorough knowledge, not only of the species, but also of the free-ranging population, to optimize management of the captive population. Such knowledge is necessarily based on interdisciplinary works combining genetics, population ecology, and behavioral ecology. For example, the study of the socio-sexual system of the species (Hingrat, Saint Jalme, Chalah, Orhant, & Lacroix, 2008; Lesobre, Lacroix, Le Nuz, et al., 2010; Vuarin et al., 2019) allows adjusting management in captivity while knowledge on the genetic status of the free-ranging population (*e.g.* population genetic structure; Lesobre, Lacroix, Caizergues, et al., 2010), its spatial structure (Hingrat et al., 2004), and the fate of individuals released into the wild (Bacon, Hingrat, & Robert, 2017; Bacon, Robert, & Hingrat, 2019; Hardouin, Hingrat, Nevoux, Lacroix, & Robert, 2015; Hardouin et al., 2014) provide useful information on the spatial scale to sample founders and on the expected genetic relationship amongst them and with the existing captive population.

ACKNOWLEDGEMENTS

The Emirates Center for Wildlife Propagation (ECWP) provided the funding and the data for this study. The ECWP is a project of the International Fund for Houbara Conservation (IFHC). We are grateful to H.H. Sheikh Mohammed bin Zayed Al Nahyan, Crown Prince of Abu Dhabi and Chairman of the IFHC and H.E. Mohammed Al Bowardi, Deputy Chairman of IFHC, for their support. This study was conducted under the guidance of RENECO International Wildlife Consultants LLC., a consulting company managing ECWP. We are grateful to Dr. Y. Hingrat, research manager, and G. Leveque, project director, for their supervision. We also tank Dr. H. Abi Hussein for her wise statistical advices, and two anonymous reviewers for their valuable comments.

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21 Rabier

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TABLES**TABLE 1. Sample sizes of pedigree and molecular analyzes of the ECWP's captive population of Houbara, for each study year.**

Group	Year	N _{pedigree}	N _{founders}	%known ancestry	AvgGen	MaxGen	N _{genotyped}	%typed
Breeding flock	1997	231	49	99.4%	1.03	2	62	26.8%
	1998	328	52	99.5%	1.16	2.25	99	30.2%
	1999	347	54	99.6%	1.22	2.25	124	35.7%
	2000	473	73	99.5%	1.31	2.5	189	40.0%
	2001	507	76	99.6%	1.46	3	356	70.2%
	2002	596	80	99.3%	1.59	3.12	523	87.8%
	2003	946	79	99.2%	1.86	3.25	753	79.6%
	2004	1 349	87	99.1%	2.06	3.25	970	71.9%
	2005	2 372	112	99.2%	2.26	3.37	1 448	61.0%
	2006	2 736	127	99.3%	2.36	3.87	1 663	60.8%
	2007	3 244	159	99.1%	2.44	4.22	2 048	63.1%
	2008	4 314	191	98.0%	2.50	4.28	2 841	65.9%
	2009	5 437	201	98.9%	2.57	4.64	3 510	64.6%
	2010	6 625	234	98.2%	2.58	4.70	4 295	64.8%
	2011	6 942	258	98.2%	2.55	4.84	4 503	64.9%
	2012	7 081	260	98.2%	2.54	4.94	4 495	63.5%
	2013	8 409	268	98.1%	2.54	4.94	5 169	61.5%
	2014	9 002	266	98.0%	2.56	5.05	5 363	59.6%
Breeding chicks	2015	8 412	268	98.1%	2.54	5.05	5 066	60.2%
	2016	8 020	262	98.0%	2.55	5.05	4 800	59.9%
	2017	8 648	262	98.0%	2.59	5.05	5 035	58.2%
	2018	8 631	278	98.1%	2.61	5.05	4 833	56.0%
	1997	165	42	100%	1.39	2.25	38	23.0%
	1998	116	40	99.6%	1.43	2.25	26	22.4%
	1999	148	53	99.3%	1.55	2.5	66	44.6%
	2000	316	74	99.4%	1.64	3	169	53.5%
	2001	266	67	99.2%	1.84	3.12	186	69.9%
	2002	530	73	99.3%	2.23	3.25	258	48.7%
	2003	529	80	98.8%	2.42	3.37	246	46.5%
	2004	1 144	106	99.3%	2.53	3.87	505	44.1%
	2005	599	114	99.4%	2.73	4.22	282	47.1%
	2006	777	143	98.4%	2.77	4.28	423	54.4%
	2007	1 362	185	98.9%	2.71	4.64	83	61.2%
	2008	1 207	174	98.3%	2.84	4.80	662	54.8%
	2009	1 847	225	96.5%	2.56	4.84	1 018	55.1%
	2010	611	224	99.0%	2.13	4.94	336	55.0%
	2011	768	236	98.5%	2.37	4.53	320	41.7%
	2012	1 690	248	97.5%	2.54	5.16	782	46.3%
	2013	1 239	243	97.8%	2.70	4.20	473	38.2%
	2014	469	229	98.6%	2.34	4.16	218	46.5%

31 Rabier

Group	Year	N _{pedigree}	N _{founders}	%known ancestry	AvgGen	MaxGen	N _{genotyped}	%typed
Surplus chicks	2015	468	237	97.7%	2.67	4.22	197	42.1%
	2016	483	244	97.7%	2.89	4.62	134	27.7%
	2017	659	261	97.8%	2.79	4.16		
	1997	13	23	100%	1.12	1.5		
	1998	12	18	100%	1.33	2		
	1999	24	32	100%	1.61	2.5		
	2000	113	64	99.3%	1.76	3		
	2001	144	60	99.0%	1.96	3.12		
	2002	247	57	99.6%	2.32	3.5		
	2003	322	64	99.5%	2.53	3.69		
	2004	979	74	99.0%	2.69	3.87		
	2005	1 444	102	99.3%	3.00	4.28		
	2006	3 088	118	99.3%	3.24	4.61		
	2007	6 784	167	99.3%	3.34	4.64		
	2008	7 025	195	99.4%	3.37	5.08		
	2009	14 759	231	98.7%	3.52	5.44		
	2010	14 376	226	98.7%	3.61	5.29		
	2011	13 966	242	98.2%	3.68	5.45		
	2012	15 572	256	98.1%	3.65	5.34		
	2013	19 101	266	98.3%	3.61	5.44		
	2014	19 956	262	97.9%	3.55	5.38		
	2015	22 885	270	97.9%	3.49	5.39		
	2016	20 761	384	97.1%	3.47	5.49		
	2017	21 545	392	98.1%	3.46	5.49		

N_{pedigree}: pedigree size; N_{founders}: number of pedigree's founders; AvgGen: average generation; MaxGen: maximum generation; N_{genotyped}: number of genotyped individuals; %known ancestry: proportion of known ancestry within the pedigree; %typed: proportion of genotyped individuals

TABLE 2. Average pedigree-based inbreeding coefficient F , average mean kinship M_k , and proportions of individuals with $F > 0.1$ of the three groups of the ECWP's captive population of Houbara, in 2017. SD is the standard deviation.

Group	N	M _k	$\bar{F}_{2017} \pm SD$	Proportion of individuals with $F > 0.1$
Breeding flock	8 648	0.008±0.003	0.003±0.012	0.2%
Breeding chicks	659	0.008±0.002	0.0004±0.003	0%
Surplus chicks	21 545	0.008±0.002	0.002±0.006	0.02%

N: sample size; M_k: average pedigree-based mean kinship in 2017; $\bar{F}_{2017}$: average pedigree-based inbreeding coefficient in 2017

32 Rabier

TABLE 3. Genetic characteristics of 7 microsatellite loci used for the molecular analyzes of all individuals that have been genotyped since 1997 (N = 7 158).

Locus	Ar	Ho	He	uHe	Significance of Hardy-Weinberg equilibrium test
A113a	25	0.83	0.82	0.82	***
A120	7	0.68	0.69	0.69	n.s.
A210	6	0.73	0.77	0.77	***
A21	7	0.41	0.40	0.40	n.s.
A29	5	0.74	0.74	0.74	***
A2	17	0.76	0.79	0.79	***
D118	9	0.66	0.66	0.66	n.s.

Ar: allelic richness; Ho: observed heterozygosity; He: expected heterozygosity; uHe: unbiased expected heterozygosity. n.s.: not significant; ***: p-value < 0.001.

TABLE 4. Results of correlation tests between pedigree-based individual inbreeding coefficient F and molecular-based individual multilocus heterozygosity MLH for different batches of all genotyped individuals of ECWP's captive population of Houbara.

Grouping	Subgroup	N	Pearson's test	
			r	significance
all genotyped individuals		7 158	-0.061	***
by sex	male	2 909	-0.074	***
	female	3 960	-0.062	***
by F interval	$F \leq 0.01$	6 138	-0.036	*
	$0.01 < F \leq 0.05$	794	-0.091	*
	$0.05 < F \leq 0.10$	150	-0.010	n.s.
	$0.10 < F \leq 0.15$	38	0.055	n.s.
	$0.15 < F$	38	-0.102	n.s.
by generation	$G \leq 2$	2 272	-0.028	n.s.
	$2 < G \leq 3$	2 727	-0.049	*
	$3 < G \leq 4$	1 929	-0.078	**
	$G > 4$	226	0.05	n.s.

N: sample size; r are the correlation coefficients of the parametric Pearson's test; n.s.: not significant; *: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001. Significance was assessed on p-values adjusted by Bonferroni method.

FIGURE LEGENDS

Fig. 1. Interannual variation of (A) the average mean kinship M_k , (B) the average inbreeding coefficient F , and (C) the proportion of individuals with $F > 0.1$, for the three groups of ECWP's captive population of Houbara. Solid lines represent breeding chicks, long dashes line represents the breeding flock, and dot dashes line represents released chicks.

Fig. 2. Interannual variation of (A) the average allelic richness A_r and (B) the observed heterozygosity of the breeding flock of ECWP's captive population of Houbara. The solid line represents H_o and the long dashes line represents the unbiased expected heterozygosity uH_e .

Fig. 3. (A) correlation between pedigree-based individual inbreeding coefficient F and molecular-based individual multilocus heterozygosity MLH when considering all genotyped individuals of ECWP's captive population of Houbara ($N = 7\ 158$). The blue line represents the linear relationship between F and MLH ($R^2 = 0.004$). (B) distribution of the number of individuals by pedigree-based inbreeding coefficient F when considering all genotyped individuals of ECWP's captive population of Houbara. The dashed vertical blue line represents the pedigree-based average inbreeding coefficient which was equal to 0.006.

RESEARCH HIGHLIGHTS

A strict genetic management within captive breeding is essential in the aim of preserving populations' genetic diversity. Discrepancies between pedigree-based and molecular analyzes show that both must be used in integrative genetic managements.

338x190mm (300 x 300 DPI)

Fig. 1. Interannual variation of (A) the average mean kinship M_k , (B) the average inbreeding coefficient F , and (C) the proportion of individuals with $F > 0.1$, for the three groups of ECWP's captive population of Houbara. Solid lines represent breeding chicks, long dashes line represents the breeding flock, and dot dashes line represents released chicks.

57x41mm (800 x 800 DPI)

Fig. 2. Interannual variation of (A) the average allelic richness Ar and (B) the observed heterozygosity of the breeding flock of ECWP’s captive population of Houbara. The solid line represents Ho and the long dashes line represents the unbiased expected heterozygosity uHe.

57x25mm (800 x 800 DPI)

Fig. 3. (A) correlation between pedigree-based individual inbreeding coefficient F and molecular-based individual multilocus heterozygosity MLH when considering all genotyped individuals of ECWP's captive population of Houbara ($N = 7\,158$). The blue line represents the linear relationship between F and MLH ($R^2 = 0.004$). (B) distribution of the number of individuals by pedigree-based inbreeding coefficient F when considering all genotyped individuals of ECWP's captive population of Houbara. The dashed vertical blue line represents the pedigree-based average inbreeding coefficient which was equal to 0.006.

57x25mm (800 x 800 DPI)