Trends in genome diversity of small populations under a conservation program: a case study of two French chicken breeds

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Abstract

Livestock biodiversity is declining globally at rates unprecedented in human history. Of all avian species, chickens are among the most affected ones, because many local breeds have a small effective population size that makes them more susceptible to demographic and genetic stochasticity. The maintenance of genetic diversity and control over genetic drift and inbreeding by conservation programs are fundamental to ensure the long-term survival and adaptive potential of a breed. However, while the benefits of a conservation program are well understood, they are often overlooked. We here used temporal whole-genome sequencing data to assess the effects of a conservation program on the genetic diversity ($\Delta\pi$), deleterious variation (ΔL), and inbreeding (ΔF) of two local French chicken breeds, the Barbezieux and Gasconne.
We showed that when the conservation program is consistent over time and does not undergo any major organizational changes (i.e., Barbezieux), the loss of genetic diversity is limited. This was true for both pedigree and genomic inbreeding, but also for the genetic load which remained limited. However, when a conservation program is interrupted or re-initiated from scratch (i.e., Gasconne), the loss of genetic diversity can hardly be limited as a result of the bottleneck effect associated with the re-sampling.
Our results reinforce the imperative to establish and sustain existing conservation programs that aim to keep populations with a relatively small effective population size from the brink of extinction. Moreover, we conclude by encouraging the use of molecular data to more effectively monitor inbreeding at the genome level while improving fitness by tracking deleterious variants.

Keywords: Conservation program, Inbreeding, Genetic diversity, Genetic load, Chicken

Introduction

Livestock breeds are recognized as important components of world biodiversity since they harbor genetic variants that can be useful to agriculture in the future. Nevertheless, livestock diversity is declining globally, as shown by the high rate of world's livestock breeds reported in *The State of the World's Biodiversity for Food and Agriculture* of the Food and Agriculture Organization (FAO) as being at risk of extinction (Scherf, Pilling, et al., 2015). Among the 7,745 local livestock breeds still in existence, 26% are at risk of extinction, while 67% are of unknown risk status (Bélanger, Pilling, et al., 2019). Many of the threats affecting livestock diversity have been identified and these include indiscriminate cross-breeding, production system intensification, and introduction/increased use of exotic breeds. The impact of these threats needs to be better assessed, particularly with respect to local animal genetic resources (AnGR) (Bélanger, Pilling, et al., 2019).

Avian species, and particularly chicken, are among the livestock species with the highest percentage of breeds with a critical status, although difference can be observed at the national and regional level; for instance, in France, of the approximately 50 officially recognized breeds, 45 are classified as endangered (E Verrier et al., 2015). The establishment in the mid 20th century of few, specialized breeding industries that rely on a few selected lines for egg (layer) or meat (broiler) production has been partially responsible for the decline in local chicken diversity in Europe and North America (Muir et al., 2008). However, the large number of chicken breeds at risk is also due to the often unclear and problematic definition of a breed, which makes any direct risk assessment rather challenging. From a genetic perspective, local chicken breeds are at major risk of extinction because their small population size makes them more susceptible to stochastic demographic and genetic events. The risk of genetic erosion is often enhanced by the lack of conservation programs, either on farm, by livestock keepers in the production system (i.e., *in situ*) or in dedicated facilities, such as ark farms or experimental facilities (i.e., *ex situ in vivo*) (Bortoluzzi, Crooijmans, et al., 2018). Furthermore, semen cryopreservation (i.e., *ex situ in vitro* conservation) is still not routinely used in chickens.

Genetic drift, or the random fluctuation in allele frequencies, is the main stochastic event responsible for the loss of genetic diversity in small populations (J Fernández, Meuwissen, et al., 2011). In fact, genetic drift can reduce the viability and adaptive potential of a population. Recent studies in wild and domesticated species (Abascal et al., 2016; Bortoluzzi, Bosse, et al., 2020; Robinson et al., 2019; Van Der Valk et al., 2019; Xue et al., 2015) have shown that the risk of extinction in small populations is also a consequence of harmful mutations that lower the fitness of an individual carrying them. The rationale is the reduced efficiency of natural selection at purging harmful mutations because of genetic drift (Kimura, 1957; Ohta, 1973). Therefore, harmful mutations can accumulate and reach fixation in the genome. Additionally, as small populations suffer from inbreeding resulting from mating between close relatives (Kardos et al., 2016), (recessive) mutations in homozygous state can express their harmful nature.

Conservation programs are able to maintain genetic diversity while controlling for genetic drift (De Cara et al., 2013; J Fernández, Meuwissen, et al., 2011; J Fernández, M Toro, et al., 2004). However, the impact of a conservation program on a population in terms of genetic diversity, deleterious variation, and inbreeding have rarely been investigated in local livestock breeds at the whole genome level. Such assessment is of particular relevance today, as the maintenance of high genetic diversity alone is not sufficient to ensure the long-term survival of populations of small size (Oosterhout et al., 2022).

Recent advances in sequencing technologies can help us in the task of evaluating a conservation program with the aim of providing objective recommendations to effective management practices for small local populations (Díez-del-Molino et al., 2018; Habel et al., 2014). Temporally sampled genomic data are a powerful tool to monitor changes in genetic parameters, including genetic diversity ($\Delta\pi$), inbreeding level (ΔF), deleterious

variation (ΔL), and, if applicable, selection (ΔS), as illustrated in the case of the Spanish cattle breed Asturiana de Los Valles (Boitard et al., 2021). Hence, when possible, temporal genomic indices should be quantified to evaluate and guide existing and future conservation programs (Díez-del-Molino et al., 2018).

In this study, we assessed the impact of 10 generations of a conservation program on the genetic and deleterious variation of two local French chicken breeds, the Barbezieux and Gasconne, by means of whole-genome sequencing data. For each breed, a conservation program was established in 2003 by the breeders' association in collaboration with a professional breeding center *The Centre de Sélection de Béchanne*, with the methodological support of the French Union of Poultry and Fish Breeders (SYSAAF) for the management of pedigree data and mating plans. However, while the conservation program of the Barbezieux continued with a one-year generation interval, that of the Gasconne was discontinued and completely replaced in 2009 with a new set of founder sires and dams, unrelated to those used in 2003. Yet, the common point between these two breeds was the very small number of founders, being less than 10 sires and 10 dams.

To assess the effectiveness of these two conservation programs at maintaining genetic variation, temporal genomic erosion between 2003 and 2013 was analyzed by quantifying delta indices related to genetic diversity ($\Delta\pi$), inbreeding (ΔF), and deleterious variation (ΔL), which were ultimately used as reference to provide recommendations for future management practices.

Material and methods

Sampling statement

Data used in this study were collected as part of routine data recording for a conservation program. Blood samples collected for DNA extraction were conducted under veterinary care for routine health monitoring and only used for the conservation program, in line with the French law on the protection of farm animals.

History of the populations

Two local chicken breeds, the Barbezieux and Gasconne, were chosen for this study because of their management history and availability of gene bank samples at two time periods. The origin of the two breeds dates back to the 19th century in South-west France in the city of Barbezieux-Saint-Hilaire for the Barbezieux and the city of Masseube for the Gasconne (Fig. 1a). Both breeds are considered as dual-purpose breeds, laying about 200 eggs per year while producing high quality meat. They are robust and are generally raised in free range. They are generally valued locally in the short chain market where they benefit from a designation of origin.

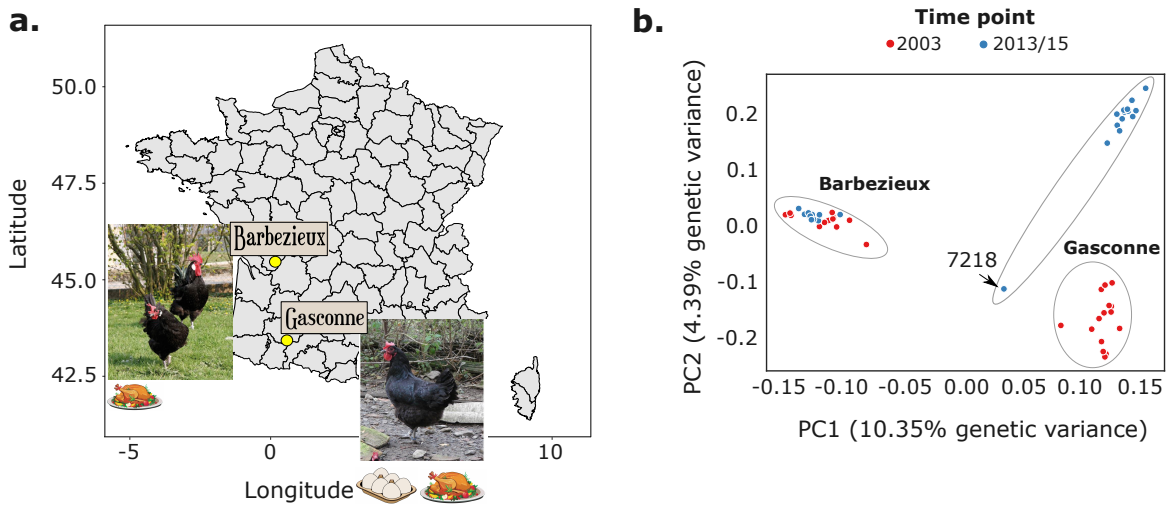
Table 1. Samples sequenced for this study

Breed name	Sample size	Sampling year	Geographic origin	City of origin	Morphology
Barbezieux	15	2003	South-West France	Barbezieux-Saint Hilaire	Simple comb, black feather
Barbezieux	14	2013	South-West France	Barbezieux-Saint Hilaire	Simple comb, black feather
Barbezieux	1 (semen)	2015	-	-	-
Gasconne	15	2003	South-West France	Masseube	Simple comb, black feather
Gasconne	14	2013	South-West France	Masseube	Simple comb, black feather

In 2003, both breeds were included in a research project aimed at defining the main parameters which determine the success and sustainability of exploitation programs for local breeds (Tixier-Boichard et al., 2006). The project started simultaneously for both breeds with the first animals born in 2003 recorded in a pedigree at the Breeding Center of Bechanne for the Barbezieux and at the Agricultural school of Saint Christophe for

the Gasconne. For each breed, a breeders' association was set up to define the breeding objectives and to monitor the management program. Thanks to the DNA bank established in the frame of the French Center for Animal Biological Resources, CRB-Anim research infrastructure (https://crb-anim.fr/access-to-collection/), we had access to samples for an equal number of individuals, for each breed, both at the start of the conservation program (i.e., founder population) and 10 generations after to get a good picture of each population (Table 1).

Figure 1. Samples and population structure. **a.** Geographic origin of the Barbezieux and Gasconne breed, with relative breeding objectives (meat or meat/egg). **b.** Principal component analysis (PCA) performed using 15,191,755 bi-allelic SNPs after filtering for a missing rate of 10%. Individuals from each breed are colored with respect to their sampling year.



Sampling

To perform a time series analysis and to monitor the impact of management practices across 10 generations, we sampled 15 founder individuals born in 2003 for each breed. Then we completed these samples with 14 individuals born in 2013 for both Barbezieux and Gasconne breeds (Table 1). In addition, we completed the Barbezieux breed sampling with the semen of one male collected in 2015, bringing the total sample size to 59. Except for this latter one, all samples consisted of DNA extracted from blood. Sibs and half sibs were discarded from the selection process to minimize relatedness in the dataset. For each breed we also obtained the following additional information: (1) complete pedigree data for the period 2002-2019 for the Barbezieux and 2009-2019 for the Gasconne; (2) body weight at 8 weeks of age from 2003 to 2019 for the Barbezieux and from 2010 to 2019 for the Gasconne; and (3) six reproductive traits for the period 2003-2018 and 2011-2018 for the Barbezieux and Gasconne, respectively, defined as the average number of eggs set in the incubation, the average number of infertile eggs, average number of hatched eggs, % fertile eggs, % hatched eggs, and late embryonic mortality.

Pedigree and phenotypic data were provided by the SYSAAF under a data transfer agreement signed with the breeders' association.

Sequencing, read processing and alignment

Sequencing was carried out on a NovaSeq 6000 sequencing machine using standard library preparation protocols. Sequencing statistics are given for each individual in Supplementary Table S1. All analyses were

based on an alignment of sequence data from all samples to the chicken GRCg6a reference genome (GenBank assembly accession: GCA_000002315.5). The alignment and variant calling pipeline were developed under the Innovative Management of Animal Genetic Resources (IMAGE) project and are publicly available (see Data availability). Briefly, sequence data were mapped to the chicken reference genome with the BWA-mem v0.7.17 algorithm (Li and Durbin, 2009), using default options. Local realignment around insertions/deletions (InDels) and base quality recalibration were carried out in GATK v3.7 (McKenna et al., 2010) to improve variant concordance and to correct for sequencing errors. SNPs and InDels calling was performed independently for each sample and by each caller (i.e., Mpileup (Li, B Handsaker, et al., 2009), Freebayes (Garrison and G Marth, 2012), and GATK GenotypeGVCFs (McKenna et al., 2010)), retaining only variants with a mapping quality $>$30 and base quality $>$10 (Supplementary Figure S1a). GATK variants were then filtered using the Variant Quality Score Recalibration (VQSR), which takes as positive training set the set of variants called by the three callers and as negative training set the unfiltered variants uniquely called by one of the three callers (Supplementary Figure S1b). Additional filtering was performed on the final VCF file, retaining genotypes whose coverage was between 4x and 2.5 the individual mean genome-wide coverage.

Principal component analysis

A principal component analysis (PCA) was carried out in SNPRelate (Zheng et al., 2012) for R v3.2.0 to detect any existing structure within and between the two breeds. The first PCA was performed on all samples, considering as input only bi-allelic SNPs with a missing rate $<$10% (n = 15,191,755 SNPs). We did not perform any linkage disequilibrium (LD) pruning to avoid excluding sites corresponding to fixed differences between the two breeds. In addition to the all-samples PCA, we performed a breed-specific PCA, in which bi-allelic SNPs were also pruned for an |LD| threshold of 0.5. After pruning, 84,930 and 108,403 SNPs remained for the Barbezieux and Gasconne, respectively.

Population differentiation was further analyzed by estimating the fixation index (F_{st}) between populations (i.e., combination of breeds and time period) in consecutive non-overlapping 50-kb windows in VCFTools v0.1.13 (Danecek et al., 2011) after removing windows with less than 300 SNPs.

Genome-wide heterozygosity

Heterozygosity was calculated for each individual separately as the corrected number of heterozygous genotypes in consecutive non-overlapping windows of 100-kb, following the approach of Bortoluzzi, Bosse, et al. (2020) based on Bosse, Megens, Madsen, Paudel, et al. (2012). Heterozygosity was calculated for the entire autosomal genome (InDels excluded). However, only windows where at least 80% of the sites met the coverage criteria (i.e., 4x $\leq$ coverage $\leq$ 2*mean genome-wide coverage) were considered for the individual genome-wide heterozygosity (Bortoluzzi, Bosse, et al., 2020; Bosse, Megens, Madsen, Paudel, et al., 2012).

Within-individual runs of homozygosity

Runs of homozygosity (ROHs), here defined as genomic regions showing lower heterozygosity than expected based on the average genome-wide heterozygosity, were identified using the approach of Bortoluzzi, Bosse, et al. (2020) based on Bosse, Megens, Madsen, Paudel, et al. (2012). To identify ROHs, we first calculated the corrected number of heterozygous genotypes in consecutive non-overlapping 10-kb windows along the genome of each individual. We then considered ten consecutive 10-kb windows at a time (i.e., 100-kb) and applied two filtering steps. First, we calculated the level of heterozygosity within the 10 consecutive windows - here indicated as πw - and retained only those for which πw was below 0.25 the average genome-wide heterozygosity - here indicated as πg. We used a threshold of 0.25 as this value was found to be able to filter out windows enriched for heterozygous sites. In the second step we tried to reduce the impact of local assembly and alignment errors as much as possible by relaxing another set of parameters within the retained 10

consecutive windows - from here onwards we will refer to these windows as candidate homozygous stretches.

Sequence data are prone to assembly and alignment errors and very often these errors result in a peak of heterozygous sites. To filter out these peaks, we first looked at each window making up the candidate homozygous stretch to identify any window whose heterozygosity was twice that of the genome (πg). If πw did not exceed 20% the average genome-wide heterozygosity also when considering windows with a peak in heterozygosity, then the candidate homozygous stretch was retained. Otherwise, the candidate homozygous stretch was discarded. All retained homozygous stretches were subsequently concatenated to form the final set of ROHs.

For each ROH, we calculated its size (i.e., the number of 10-kb windows that make up the ROH) and length (i.e., the total length of the ROH including windows that did not meet the coverage criteria). Ideally, these two measures are the same. However, this is often not the case in low coverage data. In this study, coverage was not an issue and for this reason we only discarded from further analyses 187 ROHs for which the size-to-length ratio was $<$2/3. All remaining 4,658 ROHs were classified - based on their size - into short ($\leq$ 100 kb), medium (0.1-3 Mb), and long ($\geq$ 3 Mb).

Between-individual sequence identity

To identify genomic regions shared between individuals (identity-by-descent segments or IBD), we first resolved the phase of the distinct haplotypes within each sample using Beagle v5.0 (BL Browning, Zhou, et al., 2018). Phasing was performed on the all-samples dataset of filtered variants using 10 burnin iterations, 12 phasing iterations, a window length of 20 cM, a window overlap of 2.0 cM, and an effective population size of 100,000. Phasing was performed on each chromosome separately, providing each time a genetic map with information on variants positions in cM units using the linkage map of Elferink et al. (2010). IBD segments between individuals were identified using the following parameters in the refinedIBD program (BL Browning and SR Browning, 2013): a 20 cM window length, a minimum length of 1.0 cM to report an IBD segment, and a LOD score of 3.0.

Pedigree- and genomic-based inbreeding

We used the pedigree provided by the SYSAAF to estimate the pedigree inbreeding coefficient, F_{PED}, in 43 of the 59 samples. Samples that were not present in the pedigree and were thus excluded from the F_{PED} estimation were the 15 Gasconne founders and the Barbezieux sample from 2015. In addition to the expected inbreeding, we used our set of ROHs to estimate the realized inbreeding, or F_{ROH}, here expressed as the ratio between the total length of ROHs within an individual (L_{ROH}) and the actual length of the genome (L_{auto}) covered in our dataset (n = 960,268,821 nucleotides) (McQuillan et al., 2008). Sex chromosomes and mitochondrial genome were excluded from L_{auto}.

Polarization and annotation of variants

The bias towards the alleles present in the reference sequence (reference bias) can lead to inaccurate genomic analysis. To reduce the effect of the reference bias, we polarized all alleles present in our dataset as ancestral or derived with respect to the ancestral chicken sequence reconstructed from the 4-sauropsids whole-genome alignment downloaded from Ensembl (release 95). We retained only SNPs for which either the reference or alternative allele matched the ancestral allele, while ancestral alleles that did not match either chicken allele were discarded. Polarized variants were subsequently annotated using the Ensembl Variant Effect Predictor (VEP) (release 95) (McLaren et al., 2016) and the Combined Annotation-Dependent Depletion tool developed for chicken (chCADD) (Groß et al., 2020). The VEP was limited to the annotation of protein-coding variants, whereas chCADD was used to equally annotate all variants in an individual's genome, independently

of their coding potential. Before classifying our variants into functional classes, we applied a combination of filtering steps to improve the reliability of the prediction (Bortoluzzi, Bosse, et al., 2020; Drake et al., 2006). Filtering criteria included: (1) bi-allelic variants with a call rate of at least 70%; (2) genes 1:1 ortholog between chicken and zebra finch to reduce the effect of off-site mapping of sequence reads; and (3) variants outside repetitive elements as these genomic regions are often difficult to sequence and are thus prone to errors.

Functional classes

Filtered protein-coding variants were classified, following the VEP annotation, into synonymous, missense tolerated (SIFT score $>$0.05), missense deleterious (SIFT score $\leq$0.05), and loss of function (LoF) (i.e., splice donor, splice acceptor, start lost, stop gained, and stop loss). To validate the set of variants classified as damaging (i.e., deleterious and LoF) by VEP, we assigned to each variant the Genomic Evolutionary Rate Profiling (GERP) score (Davydov et al., 2010) computed on the 34-sauropsids whole-genome alignment downloaded from Ensembl (release 97). The GERP score is a measure of sequence conservation across multiple species. Since conservation is often an indicator of strong purifying selection, GERP is an excellent predictor of fitness effects and variant deleteriousness (Huber et al., 2020). Hence, of the initial set of putative damaging variants, only those with a GERP score $>$1.0 were considered as truly deleterious.

Estimation of genetic load

Estimating an individual's genetic load based on genomic data is challenging. We therefore expressed the genetic load using two different approaches. We initially expressed the genetic load as a function of the GERP score - here called GERP load - by considering, for each individual, only damaging mutations with a GERP score $>$1.0, after re-adapting the formula presented in Orlando and Librado (2019). Finally, we estimated the genetic load as a function of the chCADD score - chCADD load - by considering, for each individual, protein-coding and non-coding variants that belonged to functional classes with an average chCADD score $>$10. The chCADD load was calculated as:

$$chCADD_i = \frac{\sum_i chCADD_i}{Nhomozygous} \tag{1}$$

where $chCADD_i$ is the score of a homozygous derived variant at genomic position i and $NHomozygous$ is the total number of homozygous derived variants in each individual's genome. Thus, the chCADD score measures variant deleteriousness and can effectively prioritize variants based on a comprehensive set of functional and evolutionary properties (Groß et al., 2020; Rentzsch et al., 2019).

Signatures of selection

Genomic regions under positive selection were identified using the new generic Hidden Markov Model (HMM) developed by Paris et al. (2019). This HMM approximates the Wright-Fisher model implementing a Beta with spikes approximation, which combines discrete fixation probabilities with a continuous Beta distribution (Paris et al., 2019). The advantage of this model over existing ones is its applicability to time series genomic data. Prior to detecting regions under selection, we estimated the effective population size (N_e) in each breed separately using the NB package (Hui and Burt, 2015) for R v3.2.0. We chose the NB package because the underlying model is also an HMM with Beta transitions. To estimate Ne, we removed SNPs with an allele frequency $<$0.20 and $>$0.80 following recommendations (Paris et al., 2019). We then applied the HMM model after which we removed SNPs with a false discovery rate (FDR) threshold of 5% as estimated in the q-value package (Storey et al., 2015) for R v3.2.0.

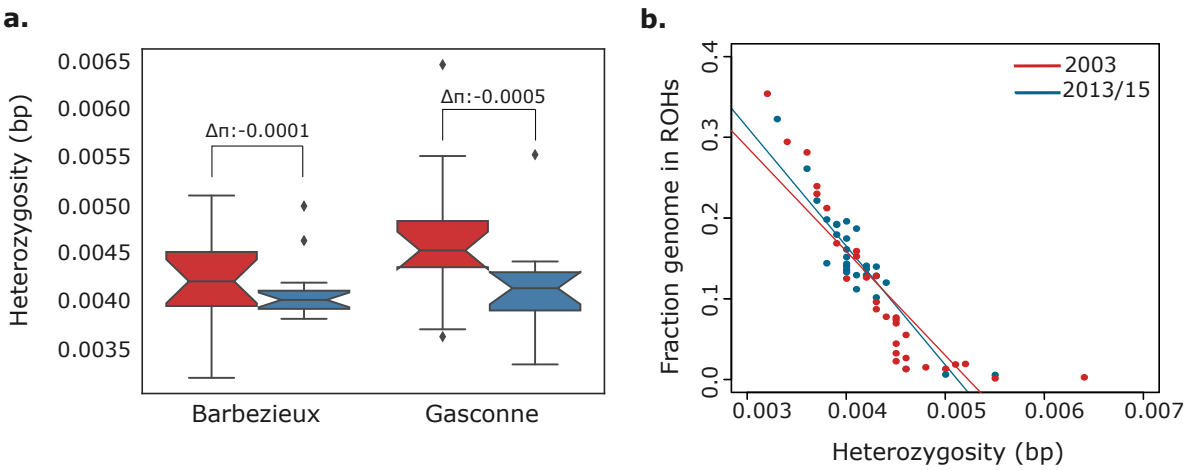
Results

We generated whole-genome sequencing data from 30 Barbezieux and 29 Gasconne birds sampled between 2003 and 2015 (Table 1). All genomes were aligned, genotyped, and annotated with respect to the chicken GRCg6a reference genome, yielding a per-individual mean genome-wide depth >10x and mapping quality >30 (Supplementary Table S1). Following variant calling and additional post-filtering steps, we identified 2 million InDels and 19 million SNPs uniformly distributed along the genome (Supplementary Table S2). Because of the limited number of SNPs on chromosomes 30 to 33, we decided to limit our analyses to the first 28 autosomes. We also excluded both sexual chromosomes and mitochondrial genome from further downstream analyses.

Temporal changes in genetic diversity and inbreeding

The separation between the Barbezieux and Gasconne samples in the principal component analysis (PCA) confirms them as genetically distinct breeds (weighted F_{st}: 0.1070) (Fig. 1b). In the all-samples PCA and breed-specific PCA (Supplementary Figure S2), we observed a clear differentiation between the Gasconne individuals sampled in 2003 and 2013 (weighted F_{st}: 0.0600), which confirms a change of the population in the 10 generations period. The result was also confirmed by the Neighbor-Joining (NJ) analysis on the identity-by-state distance relationship matrix (Supplementary Figure S3). By contrast, very little separation was observed between the two sets of birds sampled for the Barbezieux breed across the same period (weighted F_{st}: 0.0150).

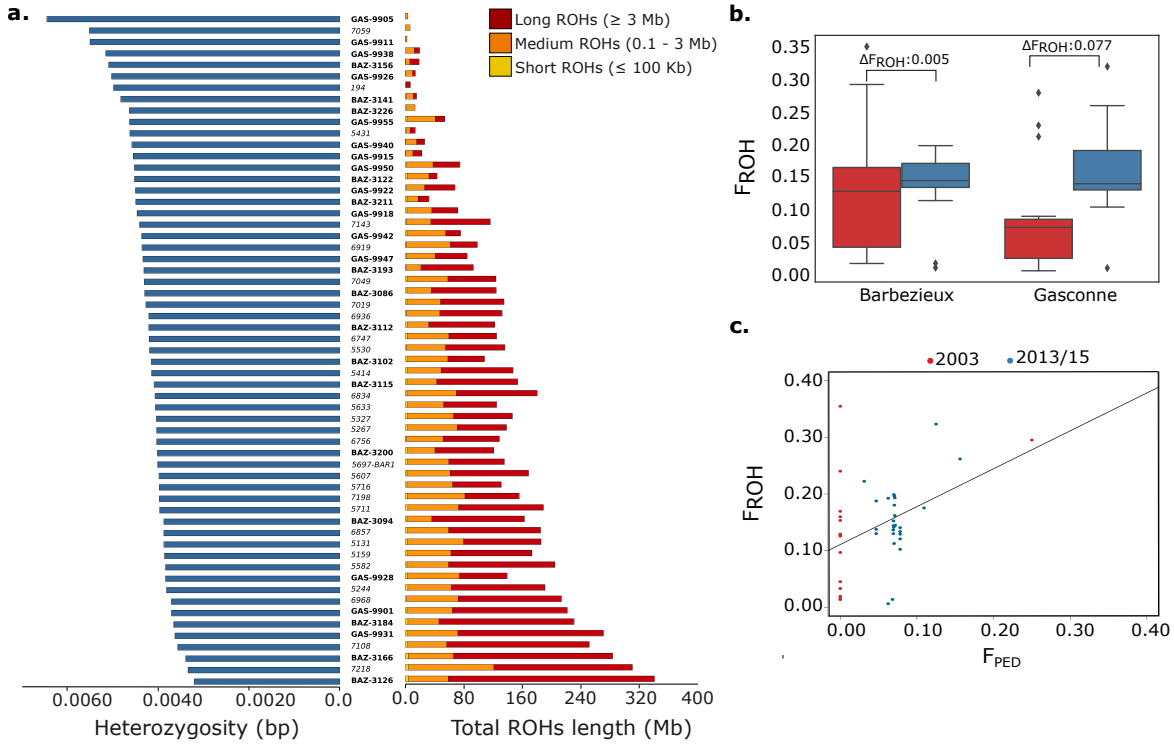
Figure 2. Temporal changes in heterozygosity. **a.** Heterozygosity is the mean autosomal heterozygosity calculated for each individual and time point along the genome in consecutive 100 kb non-overlapping windows. **b.** Correlation between individual heterozygosity (bp) and fraction of the genome covered by runs of homozygosity (ROHs).



The analysis of genome-wide heterozygosity showed that genetic diversity decreased by 2.5% (π: 4.08x10^{-3}) and 10.5% (π: 4.12x10^{-3}) in the Barbezieux and Gasconne, respectively, over the subsequent 10 years (Fig. 2a). Despite this faster decrease in heterozygosity, the Gasconne breed sampled in 2013/15 exhibited a higher within-breed diversity than the Barbezieux at the same sampling time. The within-breed reduction in genetic diversity observed in recent samples resulted from a fragmented heterozygosity distribution, where regions of high heterozygosity were interspersed by regions enriched for homozygous genotypes, also defined as runs of homozygosity (ROH). Although the mean genome-wide heterozygosity was negatively correlated with the total fraction of the genome covered by ROHs (Pearson's r: -0.90, *p-value*: 3.081x10^{-11}) (Fig. 2b), the correlation did not capture the abundance and size distribution of ROHs (Fig. 3a). Of all ROH size classes, long ROHs

($\geq$ 3 Mb) are of major concern as they result from recent close inbreeding. Barbezieux individuals sampled in 2013/15 exhibited 1 to 20 long ROHs (0.6-13% of the genome), whereas contemporary Gasconne individuals exhibited 1 to 26 long ROHs that covered up to 29% of the genome (Supplementary Table S3). Although the total number of short, medium, and long ROHs increased over the 10 generations in both breeds (Supplementary Figure S4), we observed more longer ROHs in the Gasconne than in the Barbezieux, resulting in a larger fraction of the genome in homozygous state in the former breed (Fig. 3a; Supplementary Figure S5). For each breed we also examined haplotypes shared between individuals (identity-by-descent - IBD), as these provide information on the levels of recent inbreeding. We found clear differences in the fraction of IBD segments consistent with the ROH analysis. Individuals from the Gasconne (Supplementary Figure S6c-d), and particularly those sampled in 2013, displayed a 5% higher mean level of sequence sharing than those from the Barbezieux (Supplementary Figure S6a-b), thus confirming the severe impact of recent inbreeding on the management of the Gasconne breed.

Figure 3. Runs of homozygosity (ROHs) and temporal changes in inbreeding level. **a.** Average (autosomal) genome-wide heterozygosity per individual (left) and total length of short, medium, and long ROH per individual (right). Individuals sampled in 2003 are in bold, while those sampled in 2013/15 are in italics. The prefix GAS stands for Gasconne, while BAZ for Barbezieux. Samples are ordered by decreasing heterozygosity from top to bottom.**b.** Genomic inbreeding coefficient estimated for each individual as a ratio between the total length of ROHs within an individual and the actual length of the genome covered in our dataset. **c.** Correlation between genomic inbreeding coefficient estimated from ROHs (F_{ROH}) and inbreeding coefficient estimated from the pedigree (F_{PED}).



In line with the heterozygosity and ROH analysis, the inbreeding coefficient exhibited an increase over time in the genomic, or realized, inbreeding in the two breeds (F_{ROH}), with values of delta index 15 times larger in the Gasconne (ΔF_{ROH}: 0.0776) than in the Barbezieux (ΔF_{ROH}: 0.0051) (Fig. 3b). We further calculated the pedigree inbreeding coefficient (F_{PED}) (Supplementary Table S4) and estimated the accuracy of F_{PED} in capturing individuals' relationships. Although we were able to calculate the pedigree-based inbreeding for

29 Barbezieux and 14 Gasconne samples, we found that values of F_{PED} were much more homogeneous in the Barbezieux (F_{PED}: 7%) than in the Gasconne (F_{PED}: 3-15%), confirming the trend in F_{ROH} (Fig. 3b; Supplementary Figure S7). We further correlated F_{ROH} and F_{PED} to verify the usefulness in a conservation programme of the pedigree information. As expected, we report a significant positive correlation (Pearson's r: 0.425; *p-value*: 4.92x10^{-3}) (Fig. 3c). The pedigree provided by the SYSAAF was also used to quantify changes in the number of sires and dams over the 10 generations (Supplementary Figure S8). The conservation programme was able to increase the number of breeding males and females per generation in both breeds. However, such an increase was much faster in the Gasconne, which, nonetheless, reached a total number of breeding individuals/generation lower than that of the Barbezieux (Supplementary Figure S8).

Effective population size

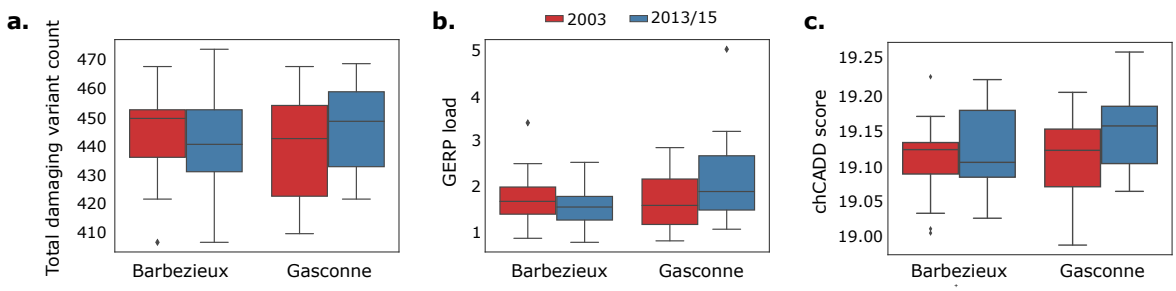
As expected from the larger size of the founding nucleus, Ne was estimated at 153.46 (CI: 145.84-161.73) in the Barbezieux, as compared to that of the Gasconne, which was estimated at 50.01 (CI: 50.00-50.08).

Temporal changes in deleterious variation

We have shown that since the start of the conservation program genetic diversity declined ($\Delta\pi$) at the costs of an increase in realized (ΔF_{ROH}) and expected (ΔF_{PED}) inbreeding, resulting from an accumulation of longer ROHs ($\geq$ 3 Mb). To verify whether the decline in $\Delta\pi$ and the increase in ΔF were associated with changes in deleterious variation, we annotated the variants with respect to their predicted impact on the encoded amino-acid into synonymous (n = 60,963), nonsynonymous (n = 24,193) and loss-of-function (LoF) (n = 337). Each filtered variant also received a chCADD score.

We first looked at the derived allele frequency (DAF) spectrum to examine the impact of purifying selection on our samples. The two breeds had more fixed, high frequency (DAF $>$0.90) benign (i.e., synonymous, tolerated) mutations than (putative) damaging (i.e deleterious, LoF) ones at the same frequency (Supplementary Figure S9). As expected, only 5% of (putative) damaging SNPs were found at very low frequency (DAF $<$0.10), as most of these mutations have been effectively purged by selection. To determine how common purging was in the two breeds, we looked at two measures of genetic load (L) (Fig. 4). We observed two opposite trends. In the Barbezieux, individuals sampled in 2013/15 displayed a net reduction in homozygous damaging mutations (Fig. 4a), resulting in a reduced average GERP load (Fig. 4b) and chCADD load (Fig. 4c). On the contrary, in the Gasconne we detected a net accumulation of homozygous damaging mutations, which resulted in an increased average GERP load (Fig. 4b) and chCADD score (Fig. 4c).

Figure 4. Genetic load and mutation burden. **a.** Total number of damaging variants identified in the dataset. **b.** Genetic load approximated using the GERP score information of each homozygous damaging mutation (GERP $>$ 1.0). **c.** Genetic load approximated from all variants independently of their functional annotation using the chCADD score.



Signatures of positive selection

The conservation program here studied was established with the objective of exploiting local breed's diversity for the production of products under quality labels. Hence, positive selection was expected to some extent. To test this hypothesis, we identified genomic regions under selection (selective sweeps) using the HMM approach developed by Paris et al. (2019). We decided to perform this analysis only on the Barbezieux, as the pedigree data of the Gasconne did not make it possible to relate the two sets of animals sampled. After filtering SNPs for an FDR threshold of 5%, no significant SNPs were identified, meaning that positive selection, if it occurred over the 10 generations, was weak enough to not leave any detectable signature in the genome. This result was further supported by the allele frequency distribution, which remained unchanged in the 10 generations (Supplementary Figure S10).

Phenotypic data: productive and reproductive performance

We analyzed one productive (Supplementary Table S5-S6) and 6 reproductive traits (Supplementary Table S7-S8) collected and provided by the SYSAAF to look at possible changes in productive and reproductive performance over the 10 generations. In the Barbezieux it seems that most of the selection effort for the trait body weight at 8 weeks took place between 2003 and 2006, where body weight was higher than in the founder generation. However, after 2006 body weight slightly decreased to 1,000g in males and 800g in females and remained rather constant up to 2013. Hence, we can conclude that over the 2003-2013 period, no clear phenotypic trend was observed for this trait.

Regarding reproduction, the % fertile eggs and the % hatched eggs increased in the Barbezieux breed, leading to a positive selection coefficient in the individuals sampled in 2013 (Supplementary Table S7). Late embryonic mortality remained rather constant in the same time period (Supplementary Table S7). Thus, the total number of chicks hatched increased because fertility had increased.

The situation in the Gasconne was quite difficult to analyze since reproductive data were only available for the 2013 generation (Supplementary Table S8), making any prior trend estimate impossible. However, when looking ahead (2013-2018), we found that all six reproductive traits have a fluctuating trend, suggesting difficulties in management and the absence of clear selection objectives.

Discussion

In this era of rapid decline in biological diversity, conservation programs have become critical for preserving the genetic diversity harbored by individual genomes (Kleinman-Ruiz et al., 2019). The importance of a conservation program on a species genome has extensively been addressed in endangered wild species (Kleinman-Ruiz et al., 2019; Robinson et al., 2019; Van Der Valk et al., 2019; Xue et al., 2015), but in local livestock breeds this has rarely been done. The rationale is that, when in place, management of local breeds cannot afford the cost of collecting high-density SNP data or, even less likely, whole-genome sequencing data. This study represents a unique case in Europe of local chicken breeds under a conservation program. Whereas the studied breeds already had SNP genotyping data for a single generation, this study is one of the few where temporal whole-genome sequencing data were used as a tool to gather critical information on the demographic and genetic processes accompanying a conservation program, with the ultimate goal of informing management and aid decision-making to keep local breeds from the brink of extinction.

How to assess the success of a conservation program

In this study, we showed that the conservation and exploitation of local breeds diversity is a valuable strategy as it allows dynamic breed conservation (É Verrier et al., 2005). The conservation program of the Bar-

bezieux and Gasconne was similar in organization and set up to that of the Bresse, a local chicken breed native to the homonymous province in eastern France (É Verrier et al., 2005). Similar to the Bresse, members of the founding nucleus were sampled from fancy breeders in the geographical area of origin of the breed, which is often defined by law. Moreover, only individuals complying with the phenotypic standard were qualified by the SYSAAF to establish the selection line at the Centre de Sélection de Béchanne. The conservation program of the Bresse has shown that when a product becomes a success, the risk status of a breed can be improved, while the loss of a breed's specific abilities can be prevented. Although in its infancy, the conservation program of the Barbezieux and Gasconne aims to achieve a similar success by linking the name of a breed to a product that has a controlled designation of origin. Despite this, the analysis of the productive and reproductive traits suggests that in both breeds more emphasis was placed on the maintenance of the breed's standards rather than on the selection for enhanced productive traits (for example, body weight). The lack of well-defined selective sweeps may also be due to the mild selection intensity that was applied. Selection pressure was all the more limited that the number of sires and dams has been increasing for the Barbezieux breed which suggests that adult fertility was a key parameter. Indeed, fertility has improved, but it could be for management reasons as well as for genetic reasons. Although our selective sweep analysis failed at identifying genomic regions under positive selection, we cannot rule out the possibility that a mild form of selection has nonetheless been taking place.

The importance of management in a conservation program

From a genetic standpoint, the primary objective of a conservation program is to maintain the highest possible levels of genetic diversity, while controlling for the increase in inbreeding. By doing so, populations will be able to respond to future changes in breeding goals and avoid a reduction in fitness (De Cara et al., 2013). As our analyses on the pedigree data clearly illustrate, conservation programs are generally founded by a small number of individuals, often coming from breeds that have a small population size themselves. Therefore, the first step in safeguarding genetic diversity is to capture as much variation as possible in the founding nucleus. This was not really the case here, where a very small number of founders were chosen for each breed. The second step is the genetic screening of the founding nucleus. Individuals selected for a conservation program may carry several genetic risks including (1) low genetic diversity, (2) high level of inbreeding, and (3) accumulated deleterious alleles. The most common practice to mitigate these genetic risks in a conservation program is the minimization of average kinship (Caballero and MA Toro, 2000; BJ Fernández and M Toro, 1999; Meuwissen, 1997), which was applied to the Barbezieux since 2003 and to the Gasconne since 2009. According to this strategy, the control over inbreeding (or co-ancestry) can be achieved if each individual contributes to the next generation with an optimal number of offspring (De Cara et al., 2013; Meuwissen, 1997). Hence, the effective population size (N_e) is maximized (Meuwissen, 1997), while the expression of (recessive) deleterious mutations is minimized. However, in order to implement this management strategy, information on individual relationships is required (De Cara et al., 2013), which is not trivial both in domesticated and wild species, but was available in the present study. The minimum kinship strategy implemented by the SYSAAF is based on the traditional analysis of pedigree data for the selection of breeding individuals. The effects of the average kinship strategy were particularly visible in the F_{PED} values of the Barbezieux, which, after an initial steep increase, stabilized at around 7%. Molecular data enabled us to take a step forward in the analysis of inbreeding, allowing us to separate the past from recent inbreeding. As a result, we were able to show that mating between close relatives (recent inbreeding), which is exemplified by the accumulation of longer autozygous segments, should be avoided as much as possible in future breeding decisions. In the case of the Gasconne, although recent inbreeding is of major concern, we cannot exclude that the recent bottleneck associated with the establishment of the conservation program in 2009 may have contributed as well. Our findings illustrate two important aspects. First, that if management is properly carried out (i.e., Barbezieux), a conservation program can still thrive even when established from a small number of founders. Hence, resampling of individuals should be carefully evaluated to limit any negative effects of changes in management

on animal genetic diversity. And second, that whenever possible, pedigree information should be recorded to elucidate management.

The role of a conservation program in purging deleterious mutations

As our study shows, in conservation programs where the population is treated as a closed nucleus, inbreeding can rapidly increase along with the probability of exposing deleterious alleles in homozygous state. A common mitigating strategy designed to restore genetic diversity and reduce inbreeding is the introduction of new individuals (and genes) from a source into a target population. In the case of the Barbezieux and Gasconne, introduction of genetic material from other breeds (introgression) is highly discouraged to preserve the genetic uniqueness of the breed. Therefore, introduction of genetic material from individuals of the same breed could offer a valuable solution to the observed loss of genetic diversity and increase in (genomic) inbreeding. High-throughput sequencing data can guide this decision, as they provide additional information on the often-neglected functional relevance of variants (Bosse, Megens, Madsen, Crooijmans, et al., 2015; Oosterhout et al., 2022).

Deleterious mutations have important consequences on an individual's survival and genetic potential. Conservation programs established without the support of molecular data are very likely to retain deleterious mutations, reducing, in the long-term, population mean fitness (De Cara et al., 2013). Deleterious mutations are a valuable source of information to perform *in-silico* prediction of fitness. Compared to previous studies that focused on protein-coding variants (Bortoluzzi, Bosse, et al., 2020; Bosse, Megens, Derks, et al., 2019; Derks et al., 2017), we here estimated genomic fitness genome-wide by focusing on all mutations independently of their coding potential. Such major breakthrough is now possible thanks to the development of the ch(icken)CADD model (Groß et al., 2020), an integrative annotation tool that can effectively score and prioritize variants genome wide. Our findings on the genomic fitness suggest that, in the case of the Barbezieux, introduction of genetic material from individuals outside the nucleus would be beneficial for the long-term conservation of the breed. However, for this management practice to succeed, individuals chosen to genetically rescue the current population should be functionally screened along with the members of the nucleus by either whole-genome sequencing data or a high-density SNP chip specifically designed for this purpose. This screening procedure should not be underestimated, as large populations with high genetic diversity may harbor recessive deleterious alleles that, if introduced in a small population, could put this population at higher risk of extinction (Bertorelle et al., 2022). While introduction of genetic material might help restore the genetic diversity in the Barbezieux, the impact of this strategy on the Gasconne is difficult to predict due to the different genetic make-up of the 2003 and 2013 founding population. We therefore recommend the SYSAAF to sequence individuals belonging to the 2009 founding nucleus in the coming years to better monitor changes in genetic diversity, inbreeding, and genomic fitness.

The added value of whole genome sequence data to assess the conservation status of a population

The Barbezieux and Gasconne breeds were included in a large-scale study aimed at comparing various indicators of genetic diversity of local chicken breeds on the basis of 57K SNP genotyping of one generation in 2013 (Restoux et al., 2022). Both breeds exhibited very similar values for all indicators that are commonly calculated (F_{it}, F_{is}, H_O, H_E, MAF, fixed alleles) and slightly different values for F_{ROH} with a higher value for the Gasconne breed, as confirmed here. Here we show that whole genome sequence data were much more efficient than SNP genotyping to reveal the differences between the two breeds, in terms of genetic history, of course, but also in terms of genetic load at a given generation. The higher resolutive power of sequencing data could be expected but the present results show that the generation of whole genome sequence data should be planned at regular intervals to better monitor the genetic status of a conserved breed.

Ex situ conservation practices in domestic animal diversity

In the context of domestic animal diversity, *ex situ* conservation practices are recognized as an essential complementary activity to *in situ* conservation actions for the maintenance of a broader genetic base. In this study, the conservation program of the Barbezieux and Gasconne relies on the maintenance of live animals (i.e., *in vivo*), though cryoconservation (i.e., *in vitro*) has been performed for one generation sampled along the program. As gene bank collections are stored for an indefinite time, they allow to preserve genetic diversity from demographic and genetic forces, such as selection and genetic drift. The interest for cryopreservation has increased over the years also for local livestock breeds, and specifically for poultry, thanks to the development of reproductive biotechnologies and efforts to enhance the use and exploitation of genetic collections (Blesbois et al., 2007). Although a gene bank is in most cases regarded as a safety collection and a complement to *in situ* and *ex situ in vivo* conservation programs, stakeholders directly involved in conservation efforts should also take advantage of existing national gene banks to regularly store genetic material for use in the future. This is particularly relevant for local breeds as their small size puts conservation programs at higher risks of failure if not properly managed and supported by molecular data, as this study shows. In the case of the Barbezieux, we encourage the analysis of the genetic material stored in the gene bank, since it may be used to reintroduce lost diversity. In the case of the Gasconne, the semen stored after 2009 is likely insufficient to reintroduce diversity. Hence, the sustainability of the conservation program would benefit, once again, from additional sequencing.

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Author contributions

M.T.B conceived the study and organized the data collection with the breeders and SYSAAF. C.B. designed the study, carried out the genomic analyses, and wrote the manuscript. R.R., B.D., and F.P. collected and provided the pedigree and phenotypic data. G.R., M.B., and M.T.B jointly supervised the study and contributed to the writing of the manuscript. All authors revised and approved the final version.

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Conflict of interest disclosure

The authors declare that they have no competing interests.

Data, script, code, and supplementary information availability

Script and codes used to identify ROHs are available online at https://github.com/cbortoluzzi/ROHs. The sequencing pipeline is available online at https://forgemia.inra.fr/bios4biol/workflows/tree/master/Snakemake/. Raw data of the 59 individuals sequenced for this study are currently being archived in the European Nucleotide Archive (ENA) under BioProject PRJEB72503 (https://www.ebi.ac.uk/ena/browser/view/PRJEB72503). All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials available at DOI 10.5281/zenodo.10691768.

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