

Genetic characterization of French local chicken breeds

G. Restoux¹, X. Rognon¹, A. Vieaud¹, D. Guémené², G. Chiron³, F. Petitjean⁴, F. Seigneurin², A. Vasilescu⁵ & M. Tixier-Boichard¹

¹ GABI, INRA, AgroParisTech, Université Paris-Saclay, Domaine de Vilvert, 78352 Jouy En Josas, France,

² SYSAAF, Unité de Recherches Avicoles, Centre INRA Val de Loire, 37380 Nouzilly, France

³ ITAVI, 23 rue Jean Baldassini, 69364 Lyon Cedex 07, France

⁴ Centre de Sélection de Béchanne, Hameau de Béchanne, 01370 Saint-Etienne-Du-Bois, France

⁵ LABOGENA - Bat 224, Domaine de Vilvert, 78353 Jouy en Josas, France

gwendal.restoux@agroparistech.fr (Corresponding Author)

Summary

The small population sizes of French local breeds raise the question of their *in situ* or *ex situ* conservation. For that goal the characterization of their genetic diversity with molecular tools appears as a preliminary key step. A total of 22 local breeds and 4 commercial lines with an average of 60 individuals per breed were genotyped using a 57K DNA chip leading to a total sample of 26 breeds and 1517 individuals. The commercial lines used as control populations included 2 broilers lines from the AvianDiv collection, one French ‘label’ slow-growing line and one brown-egg line.

Within breed genetic diversity was good but variable (mean F comprised between 3 and 28%), with inbreeding coefficients being negatively related to the size of the selection kernel. Among breed diversity was large ($F_{st}=0.25$) allowing for clear genetic identification of breeds. There was no evidence for admixture with commercial broilers but admixture could not be ruled out in the case of one local breed and the brown-egg commercial line.

Relationships among breeds were consistent with their history (origin, breeders) or usage (broilers, layers...). Finally, combining these genetic analyses with morphological data could help in detecting genomic regions of interest in a selection perspective. To conclude, French local breeds appeared to be genetically and morphologically diverse making them a good example of a successful management by both breeders and selection centers. Nevertheless attention should be paid on them for long term conservation.

Keywords: Diversity ; conservation ; management ; SNP ; Chicken

Introduction

Chicken was early domesticated and then was subject to intense selection to create breeds in order to meet expectations of the market (Tixier-Boichard *et al.*, 2011). In particular, selection of poultry led to the creation of distinct breeds specialized for either egg (layers) or meat (broiler) production since growth and reproduction traits are antagonistic (Fairfull & Gowe, 1990). Commercial lines, which are under the strongest selection, exhibit low levels of within-breed genetic diversity with a lack of rare alleles relative to ancestral populations (Muir *et al.*, 2008). These depauperate populations raise the question of sustainability in terms of genetic gain. In particular the expected global changes should lead to new breeding

goals regarding climate, resources or diseases (Hoffmann, 2010).

Genetic diversity is essential to conserve an adaptive potential necessary for sustainable livestock production (Notter, 1998). Muir *et al.* (2008) propose to combine different chicken breeds to limit the loss of genetic diversity even if diversity recovery could be limited. They suggest that combining other breeds than commercial pure lines is a promising solution in terms of genetic conservation. While wild relative species could be too distant genetically from the breeds used in selection programs, local, native or traditional chicken breeds constitute a valuable reservoir of genetic diversity (Hoffmann, 2010). Indeed they often exhibit higher and distinguishable levels of genetic diversity from commercial breeds (Weigend & Romanov, 2001, Berthouly *et al.*, 2008, Mahammi *et al.*, 2015). Consequently their conservation is critical to cope with the future challenges of livestock production.

Population sizes (*stricto sensu*) of local breeds are often limited making them prone to suffer from strong genetic drift. As a result of competition against more productive breeds they are very likely to have experienced drastic and recent bottlenecks due to a decrease of their use. Consequently many of the traditional breeds are at the edge of extinction (Davilla *et al.*, 2009). Although their management is a major concern, it appears to be difficult because small selection kernel reduce the latitude of possible actions, for example to avoid inbreeding. As a consequence a systematic survey of the genetic diversity of local chicken breeds with respect to both within and among-population levels is necessary to design efficient conservation programs whether *in-* or *ex-situ*.

In this study, using 57k SNP genotypes we analyzed 22 of the 40 French local breeds officially declared in addition to 4 commercial lines to address the following questions : i) What is the level of genetic diversity within each breed ? ii) What can explain the observed differences ? and iii) What are the genetic relationships between breeds ?

Material and methods

Sampling

22 French local breeds were selected on the basis of a primary survey looking at their threatened status and the fact they are or not part of a management program. The goal was to sample 60 individuals for each breed avoiding close relationship between individuals on the basis of pedigrees (for those from Bechanne station) or by sampling different breeders (for Hergnies, Contres and Le Mans). For some breeds different color lines were sampled (Marans). A material transfer agreement was signed between the breeders or the representative of each breed and INRA-GABI lab. DNA was extracted from blood samples by the @Bridge platform (INRA, Jouy en Josas). Finally the DNA from 4 commercial lines were added : a brown egg layer (Hendrix Genetics), a slow growing broiler (Sasso), and 2 pure line broilers (Cobb and Ross). The 2 last breeds were initially sampled during the AvianDiv project. The list of the breeds was given as a supplementary table.

Genotyping

Genotyping was done using the Illumina Infinium 60K chip developed for chicken. Analyses were made at the Labogena lab. 1517 individuals were genotyped with a total of 57636 SNPs. Genotypes of the brown egg layer breed were kindly provided by Hendrix Genetics. The Sasso genotypes resulted from a QTL detection project conducted in collaboration with the URA-INRA lab (Tours, France).

Analyses

All analyses of raw data were done using the Plink 1.9 software (Chang *et al.*, 2015). First filters based on call rates were applied in order to eliminate all loci and individuals with more than 10% of missing data (1486 loci and 15 individuals). Then loci with minor allele frequencies (MAF) lower than 1% were also removed from the dataset. Finally due to tracking problems 1 breed (Grise du Vercors, 61 individuals) and 1 individual from the Barbezieux breed were removed from the analysis. Finally the dataset was composed of 1440 individuals and 53246 SNPs with a mean call rate of 99.4%.

For each breed the MAF, the individual observed heterozygosity (H_o), the proportion of fixed alleles and individual inbreeding coefficients were computed. These latter metrics were computed either with respect to the whole population allele frequencies similarly to F_{IT} (F1) or only considering the within breed allele frequencies similarly to F_{IS} (F2). Graphics were made using the R programming language (R Core Team, 2017) and the ggplot2 package (Wickham, 2009). Unrooted neighbor-joining trees were done using the APE package (Paradis *et al.*, 2009 ; Popescu *et al.*, 2012) based on Hamming distances computed from an identity by state matrix for the whole dataset and specifically for the Marans breed.

Results

Within breed genetic diversity was globally large (figure 1) whatever the considered index with mean MAF of about 21.1% (sd=3.0%), 16.5% of the alleles were fixed within breed (sd=9.4%) and average observed heterozygosity was 32.5 (sd=2.3%). Inbreeding coefficients were very contrasted with an average of 27.8% (sd=8.7%) for F1, and an average of 3% (sd=6.0%) for F2. The average F_{ST} among breeds was 0.25. Neighbor-joining trees of all breeds were presented in figure 2-A. Trees of the Marans breed were also computed and presented in figure 2-B and 2-C.

Discussion

Differences among breed in terms of genetic diversity can result from husbandry and management practices. For instance both MAR and BGB22 had large F1 inbreeding coefficients while only MAR had also a large F2. For BGB22 the global level of genetic diversity was low due to an initial small selection kernel consisting of few individuals. This led to potentially strong bottleneck and subsequent genetic drift revealed by the high proportion of fixed alleles. However the standing diversity was well managed by appropriate mating plans as revealed by large F2 and heterozygosity. On the the other hand, MAR breed was managed by multiple breeders and consisted of multiple lines (differing in terms of colors). This within breed genetic structure resulted in an overall departure from Hardy-Weinberg equilibrium (Wahlund effect), and a subsequent large F2. F2 is much more sensitive to the structure of the breed while the F1 is a good indicator of the loss of diversity relative to ancestral population (Muir *et al.*, 2008). The rank of breeds in terms of genetic diversity was comparable with the results of a previous study by Berthouly *et al.* (2015) even if they used microsatellites leading to much more alleles and subsequent heterozygosity.

The global genetic differentiation among breeds was large, indicating that the 57K SNPs chip was appropriate to assign individuals to the right population. Moreover this indicated that the breeds were well managed trying to stay distinct from each other.

Nevertheless the genetic proximities of breeds were congruent with i) their past histories as for instance for BGB11 and BGB22 which derived from the same line, ii) their geographical origins as for CHA and CNF (Massif Central region) or GAT and BOU (Centre region), or iii) their use as for all commercial broilers consisting in a group (Cobb, Ross and Sasso) or for layers (Marans and Hendrix Genetics).

Finally the Marans breed revealed a strong genetic structure due to color variations. On the other hand this was overpassed by the breeders effect leading to contrasted management of the poultry flocks including more or less extensive exchange at the Marans network level. Indeed the third breeder had his 3 breeds clearly separated from the others while they were distinct each other. Reversely the MNC color variety was bred by two breeders. Meaning that we can obtain similar phenotype lines on the basis of two different genetic basis. This can be of interest in terms of resources for potential crossings in order to bring some genetic diversity.

Conclusion

The French local chicken breeds were very diverse and well managed with acceptable level of within population diversity. Moreover the genetic diversity was largely due to among breed differentiation. Thus the management of local breeds as a whole could be a valuable reservoir of genetic diversity for future breeding goals. In addition, such a survey will give clues for conservation programs by detecting threatened breeds and their link with other ones in the perspective of rescuing or *ex situ* conservation.

Acknowledgments

This project named BiodivA was supported by the CASDAR program of the French ministry of agriculture and the CRB-Anim infrastructure covered part of the genotyping costs.

References

- Berthouly, C., Bed'Hom, B., Tixier-Boichard, M., Chen, C. F., Lee, Y. P., Laloë, D., ... & Rognon, X. (2008). Using molecular markers and multivariate methods to study the genetic diversity of local European and Asian chicken breeds. *Animal genetics*, 39(2), 121-129.
- Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*, 4:7.
- Dávila, S. G., Gil, M. G., Resino-Talaván, P., & Campo, J. L. (2009). Evaluation of diversity between different Spanish chicken breeds, a tester line, and a White Leghorn population based on microsatellite markers. *Poultry science*, 88(12), 2518-2525.
- Fairfull, R. W., & Gowe, R. S. (1990). Genetics of egg production in chickens. *Developments in Animal and Veterinary Sciences (Netherlands)*.
- Hoffmann, I. (2010). Climate change and the characterization, breeding and conservation of animal genetic resources. *Animal genetics*, 41(s1), 32-46.
- Mahammi, F. Z., Gaouar, S. B. S., Laloë, D., Faugeras, R., Tabet-Aoul, N., Rognon, X., ... & Saidi-Mehtar, N. (2016). A molecular analysis of the patterns of genetic diversity in local chickens from western Algeria in comparison with commercial lines and wild jungle fowls. *Journal of Animal Breeding and Genetics*, 133(1), 59-70.
- Muir, W. M., Wong, G. K. S., Zhang, Y., Wang, J., Groenen, M. A., Crooijmans, R. P., ... &

- Jungerius, A. (2008). Genome-wide assessment of worldwide chicken SNP genetic diversity indicates significant absence of rare alleles in commercial breeds. *Proceedings of the National Academy of Sciences*, 105(45), 17312-17317.
- Notter, D. R. (1999). The importance of genetic diversity in livestock populations of the future. *Journal of animal science*, 77(1), 61-69.
- Paradis, E., Claude, J. and Strimmer, K. (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20, 289–290.
- Popescu, A.-A., Huber, K. T. and Paradis, E. (2012) ape 3.0: new tools for distance based phylogenetics and evolutionary analysis in R. *Bioinformatics*, 28, 1536-1537.
- R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Tixier-Boichard, M., Bed'hom, B., & Rognon, X. (2011). Chicken domestication: From archeology to genomics. *Comptes rendus biologiques*, 334(3), 197-204.
- Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2009.
- Weigend, S., & Romanov, M. N. (2001). Current strategies for the assessment and evaluation of genetic diversity in chicken resources. *World's Poultry Science Journal*, 57(3), 275-288.

Figure 1. Barplot of the different within-breed genetic diversity metrics : F1 and F2 the inbreeding coefficients respectively based on whole or within population allelic frequencies, the proportion of fixed alleles, the observed homozygosity, Ho, and the minor allele frequency (MAF).

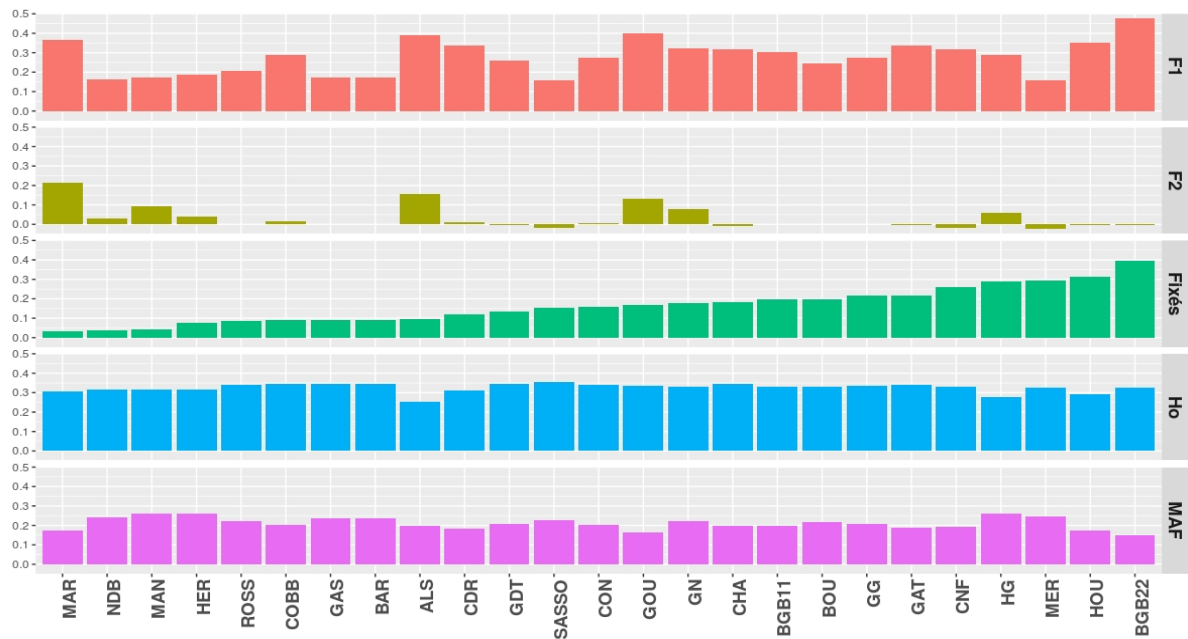
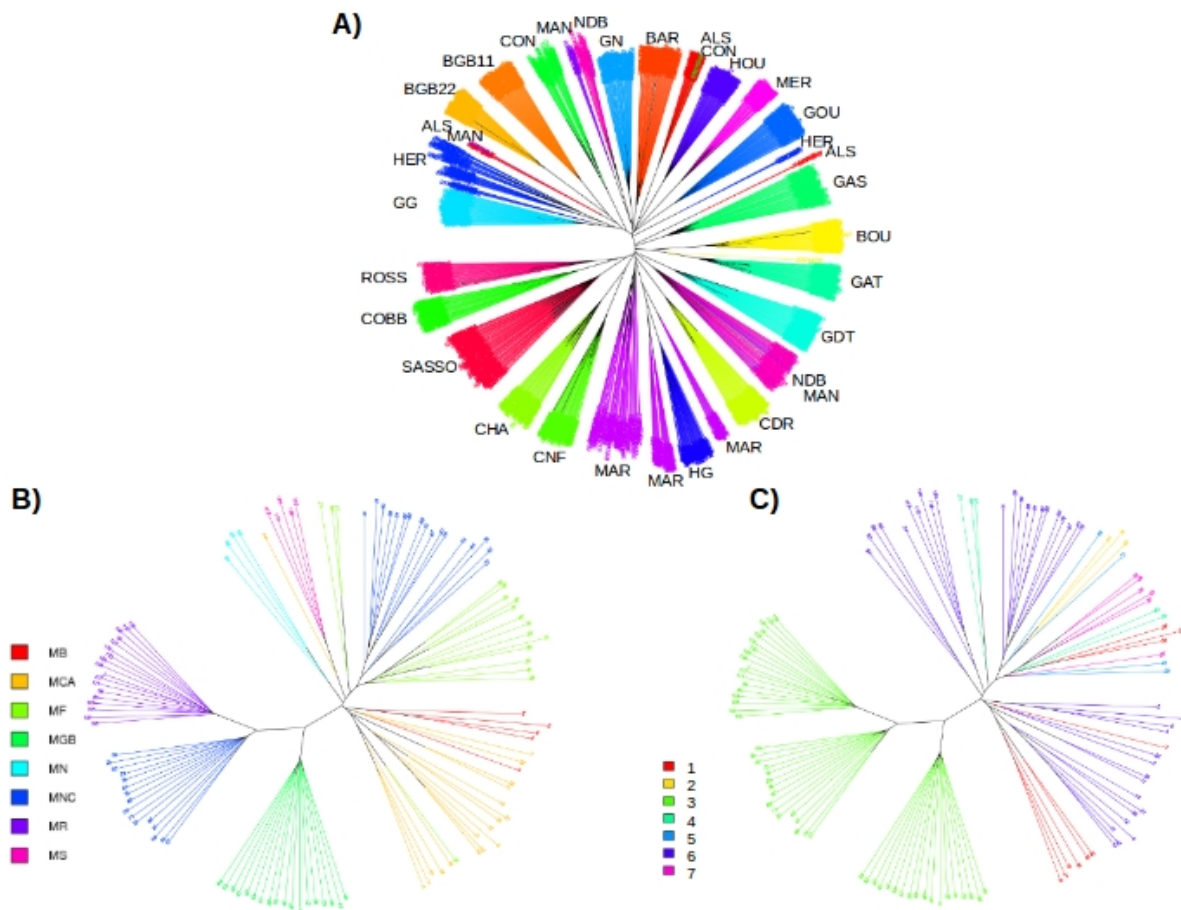


Figure 2. Unrooted neighbor-joining trees based on similarity matrices. A) Whole breeds, B) Marans breed with colors as the different lines and C) Marans breed with colors as the different breeders.



Supplementary material 1. *List of the sampled breeds.*

BREEDS	Samplin g size	Owners / Breeders / Selection centers
Alsacienne (ALS)	34	Association des Eleveurs de Race Poule d'Alsace, AERPA, représentée par Gilbert Schmitt
Barbezieux (BAR)	60	Association pour la sauvegarde de la Poule de Barbezieux, ASPOULBA, représentée par Gilbert Marchand
Bourbonnaise (BOU)	58	Comité interprofessionnel du poulet Bourbonnais, CIPB, représenté par François Périchon
Bresse Gauloise Blanche (BGB11)	60	Centre de sélection de Béchanne, représenté par Albert Thiévon
Bresse Gauloise Blanche à crête pâle (BGB22)	60	Centre de sélection de Béchanne, représenté par Albert Thiévon
Charollaise (CHA)	56	Centre de sélection de Béchanne, représenté par Albert Thiévon
Contres (CON)	42	acquies Berger, Eric Nadan, Philippe Vasseau
Coucou de Rennes (CDR)	57	Association des producteurs de Coucou de Rennes, représentée par Paul Renault
Cou-Nu du Forez (CNF)	59	Centre de sélection de Béchanne, représenté par Albert Thiévon
Gasconne (GAS)	60	Association la Poule Gasconne, représentée par Jean-Paul SERRES Le Parc Naturel Régional du Gâtinais français, représenté par Jean-Jacques Boussaingault
Gâtinaise (GAT)	58	
Gauloise Grise (GG)	60	Centre de sélection de Béchanne, représenté par Albert Thiévon
Gauloise Noire (GN)	58	Centre de sélection de Béchanne, représenté par Albert Thiévon
Géline de Touraine (GDT)	60	Syndicat Interprofessionnel de la Géline de Touraine, puis Centre de Sélection de Béchanne
Gournay (GOU)	58	Club pour la sauvegarde des Races Avicoles Normandes, CRAN, représenté par Bruno Lomenède
Grise du Vercors (GDV)	61	Association Quantia Grise du Vercors, représentée par Evelyne Tezier Reinhold Adolphi, Damien David, Roland Delvigne, Franck De Sousa, Bernard Dupas, Jean-Luc Malpoux
Hergnies (HER)	60	
Houdan (HOU)	58	Centre de sélection de Béchanne, représenté par Albert Thiévon
Le Mans (MAN)	30	Eleveurs Gwénaël L'huissier, Raymond Tertrin Jacques Beneteau, Laurent Galliot, Alain Gauguet, Eric Mandon, Daniel Mulon, Georges Riga, Dominique Tourneur
Marans (MAR)	118	
Merlerault (MER)	38	Centre de sélection de Béchanne, représenté par Albert Thiévon
Noire du Berry (NDB)	62	Le Club Français de la Poule Noire du Berry, représenté par Francis Lasne
Brown-egg layer (HG)	58	Hendrix Genetics
Label (SASSO)	96	SASSO
Cobb (COBB)	46	AvianDiv, Cobb
Ross (ROSS)	50	AvianDiv, Ross