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Insight in the genetic diversity of wild and domestic Gallus.

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Abstract

A set of 54 wild fowls from the 4 species of the genus *Gallus* and 702 domestic chickens from 24 populations were genotyped for the 57K Illumina SNP chip. Principal component analysis and phylogenetic analysis using euclidian distance were performed. Wild and domestic *Gallus* clustered in different groups, except for one H'Mong chicken. *G. gallus* was clearly separated from the other 3 species. Clustering of domestic chickens was consistent with their known selection history and F1 crossbred were easily detected. These data provide a reference for further diversity analysis by resequencing.

key-words: wild gallus, domestication, SNP, phylogeny

Introduction

Domestication of the chicken and subsequent selection has resulted in an impressive diversity in breeds, from local breeds to commercial lines. *Gallus gallus* is considered as the major ancestor of the domestic chicken but three other wild species are known for the genus *Gallus*: *Gallus sonneratii* (grey junglefowl), *Gallus varius* (green junglefowl) and *Gallus lafayetii* (Sri Lanka junglefowl). The yellow skin mutation was shown to originate from *G. sonneratii* (Eriksson et al., 2008) and gene flow was suggested between *G. sonneratii* and *G. lafayetii* (Nishibori et al., 2005). Whole-genome studies make it possible to explore chicken domestication at an unprecedented resolution. This paper presents the first step of such an exploration, based upon genotyping populations of wild and domestic fowls with a highdensity SNP chip, before resequencing the genome of a subset of individuals.

Material and methods

Animals

A set of 54 wild birds representing the 4 junglefowl species (*G. gallus, G. sonneratii, G lafayetii* and *G. varius*) was obtained from various origins : Thailand (10 *G.g. gallus* and 10 *G.g. spadiceus* from EU project Aviandiv), zoological gardens from France (6 *G.g. bankiva*; 5 *G. sonneratii*; 4 *G. lafayetii*), Vietnam (9 *G.g. spadiceus*), Taiwan (2 *G.g. bankiva*; 2 *G. sonneratii*; 3 *G. Varius*), and Japan (1 *G. sonneratii*; 1 *G. lafayetii*; 1 *G. varius*). Genotyping data from various INRA research projects (685 chickens) were re-used to obtain a representative set of experimental lines (layers or broilers), local breeds, village chickens and commercial lines (Table 1). An additional set of 17 domestic chickens from various local breeds (1 bird per breed), initially chosen for whole-genome resequencing, was included.

Genotyping

High-quality DNA was extracted from whole blood. Genotyping of the 54 wild *Gallus* and all domestic chickens was performed by LABOGENA with the 57K SNP Illumina iSelect chicken array (http://www.illumina.com/) previously used for diversity or QTL studies.

Methods

Raw data were filtered to exclude birds with low (<95%) SNP calling rate and SNPs that were either invariant across all or not called correctly for some birds. Data cleaning resulted in a total of 742 birds typed at 55759 SNPs. Genotypes were transformed to 0/1/2 vectors by

recoding each marker with 0/1 alleles: 0-allele and 1-allele were chosen randomly for each marker, not based on allele frequencies. This choice does impact downstream analyses. A pairwise distance matrix of all birds was then computed using euclidian distances between recoded genotypes. All evolutionary analysis were based on this distance matrix. Principal component analysis (PCA) was performed to visualize population separation in the genotype space. Phylogenetic analysis was done by computing a clustering tree (hierarchical clustering with average link) of all birds using the hclust function of R (R development core team 2014) and Neighbor-Joining (NJ) tree (Saitou and Nei, 1987) using the R ape package v3.0-11 (Paradis and Strimmer, 2004). The distance matrix was also used to compute a neighbor-net, using default settings of SplitsTree4 (Huson and Bryant, 2006).

Results and discussion

A decreasing calling rate was observed for *G. sonneratii*, *G. lafayetii* and *G. varius*, which is consistent with the known phylogenetic relationships between *Gallus* species, and the construction of the SNP panel, which was based upon informativeness in commercial chickens. The first two axes of the PCA accounted for 13% of total variance and the first principal plane separated the populations: the first axis discriminated domestic populations from White Leghorns to broilers, whereas the second axis discriminated the *G. gallus* versus the other *Gallus* species (figure 1). The 17 birds to be sequenced were well scattered along the first axis (black dots). The PCA confirmed that the wild *Gallus* sampled in zoological gardens had not been subjected to uncontrolled crossing with domestic birds.

Both trees (not shown) and the neighbor-net (figure 2) were quite consistent with each other. Individuals were mostly clustered by populations, which form monophyletic clades: deep inner branches are short whereas pendant ones are long. The results are expected and compatible with recent separation and low effective population sizes. Both wild *Gallus* species (*G. lafayetii, G. sonneratii, G. varius*) and *G. gallus* (gallus, bankiva, spadiceus) were separated from domestic populations, with the exception of one H'Mong chicken from Vietnam, (data not shown), this is consistent with previous evidence of introgression of wild *G.gallus* into H'Mong chickens shown with a different sampling (Berthouly et al., 2009).

Clustering of domestic chickens was consistent with their known selection history. All white Leghorn lines were connected to a common network, whatever the experimental selection performed. The situation of the DJ line was surprising, since a subset of 4 animals was clearly differentiated from the other 8. Actually, these 4 animals were crossbred between the DJ line and a commercial brown-egg line, for a QTL detection project, thus their F1 status was clearly identified by the analysis. Similarly, the F1 of the coccidiosis QTL cross between Fayoumi and one RI Leghorn line logically exhibited shared branches with each of the F0 parental line. The F0 of the RI Leghorn lines represented a small subset of the whole variation in these lines. For each parental line, either Fayoumi or RI, one individual was found at the margin of the line cluster. The SALM design corresponded also to a F0/F1 set of parents, but each F0 exhibited much less variation than that observed in the Fayoumi x RI Leghorn cross, which was expected since the SALM line were more inbred. The SALM lines showed rather long branches, but the longest branches were found with the old broiler lines and the RR line, which have been managed as closed populations of limited size, since more than 20 generations, and have undergone both inbreeding and genetic drift. The modern broiler lines, either commercial or experimental, were close to each other but not intermingled. Similarly, the experimental and commercial label lines clustered in two neighboring groups. Each local breed corresponded to a homogenous group; some of them showed a longer inner branch (i.e. Jaerhoens, Houdan), suggesting a higher inbreeding level than others (Orlov or Gournay). Regarding the unselected local chickens, either from Africa, Middle-East or Iceland, all individuals differentiated quickly and no within-population structure could be observed.

In conclusion, results were consistent with population history and allowed the detection of crossbreeding, which can be useful for the management of poultry lines and conservation programmes. The markers of the 57K array were chosen according to polymorphisms of domestic populations and did not to capture all the genetic diversity of *G. gallus*. Further analyses will be done by resequencing and will not be affected by this bias of SNP selection.

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Code	Name	population type	country	Number
BR1	Broiler 1	Broilers	not available	50
BR2	Broiler 2	Broilers	not available	46
GM	exp. broiler line	Broilers	France	54
X33	old broiler line	Broilers	France	6
X44	old broiler line	Broilers	France	6
RR	exp. RIR line	brown-egg layer	France	9
F1-COC	F1 FAY x RI	Crossbred	France	6
FAY	Fayoumi	local breed	Egypt	34
F0-FAY	Fayoumi F0 parents	local breed	Egypt	6
GOU	Gournay	local breed	France	58
HOU	Houdan	local breed	France	58
JAER	Jaerhoens	local breed	Norway (AvianDiv)	34
ORL	Orlov	local breed	Russia (AvianDiv)	34
SEB	Sebright	local breed	France	1
LAB	commercial label line	slow-growing label line	France	10
DD	exp. label line	slow-growing label line	France	12
BED	Bedouin	unselected local chickens	Israel (AvianDiv)	34
ICEL	Icelandic Landrace	unselected local chickens	Iceland (AvianDiv)	34
CIV	Village chickens	unselected local chickens	Côte d'ivoire	34
DJ	exp. Leghorn line	white-egg layer	France	12
GAVO	exp. Leghorn line	white-egg layer	France	6

 Table 1- Names and origins of the 24 domestic populations genotyped with the 60 K

RI	exp. Leghorn lines	white-egg layer	France	105
SALM	exp. Leghorn lines	white-egg layer	France	30
F0-LEG	F0 parents (RI line)	white-egg layer	France	6
24 populations		7 types		685

Figure 1 : Projection of the populations on the first plane of the Principal Component analysis. Black dots represent the 17 individual chickens that will be sequenced.

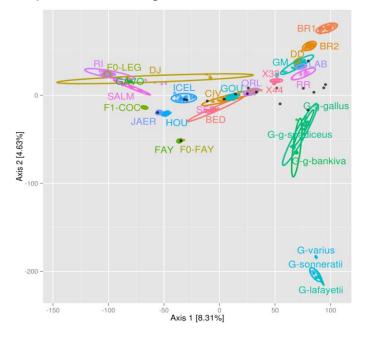


Figure 2: Neighbornet graph for both wild and domestic fowls, based upon the euclidian distance. Black dots represent the 17 individual chickens that will be sequenced

