L11 Genome-wide analyses of genetic diversity and phylogenetic relationships in the Synbreed Chicken Diversity Panel

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Abbreviated title: Genome-wide diversity in chickens

Summary

Domestic chickens show massive phenotypic and genetic variation. This can be used to quantify relationships between breeds and to detect genomic regions influencing trait divergence. Under the umbrella of the SYNBREED project, an ongoing research project supported by the German Federal Ministry of Education and Research, molecular and phenotypic data of a wide range of chicken breeds have been collected over the last years. Collection was augmented by samples from previous international cooperation projects, in particular the EC project AVIANDIV and other bilateral activities. The "Synbreed Chicken Diversity Panel (SCDP)" encompasses more than 160 diverse chicken breeds from 25 countries around the world including two wild chicken populations and commercial purebred lines. Individuals were genotyped with the newly available Affymetrix Axiom® Genome-Wide Chicken Genotyping Array, and individual phenotypic records were collected for a large part of these individuals. In this report, we provide first insight into the diversity of a subset of 82 chicken breeds of this wide spectrum, mainly sampled in Germany, by evaluating phenotypic variation and genetic relationships. Results presented here illustrate the usefulness of this resource for high resolution analyses of phenotypic variability, architecture of the genome across divergent populations, and for getting a deeper insight into phylogenetic aspects of breed development.

Keywords: Chickens, diversity, genome-wide SNP markers, phenotypic variation

Introduction

Genetic diversity within a species is displayed by a large number of breeds and populations which exhibit a wide range of characteristics and variants. Major forces creating genetic differences between breeds and populations are mutation and recombination, along with genetic drift, natural selection and breeding imposed by man. Since domestication man has strongly forced the accumulation of genetic differences between breeds and populations by isolating them from others and selecting for favorable traits. In Europe, a systematic and organized breeding in chickens has started in the second half of the 19th century. Since this time, chicken breed standards have been developed for many breeds. Selection by hobbyists

aimed at achieving an ideal picture of the phenotype in accordance to breed standards. As a consequence, in many breeds there is very little variation between individuals anymore in those traits breeds have been selected for. However, there is a wide variation of phenotypic traits between breeds and color variants making this source of variation extremely useful for discovering genetic mechanisms determining phenotypic variants. However, this has been hindered for many years by the lack of molecular markers in sufficient density across the chicken genome. Since first publication of the chicken genome sequence in 2004 (Hillier *et al.*, 2004), molecular methods and tools have become available, opening new possibilities for gene mapping. There is an increasing number of publications describing the molecular discovery of genetic mechanisms which determine phenotypes in chickens as for example the appearance of comb shape, skin color or polydactyly to name a few (Wright *et al.*, 2009; Dorshorst *et al.*, 2010; Rubin *et al.*, 2010). Recently, high density Affymetrix Axiom® Genome-Wide Chicken Genotyping Array has become available comprising more than 580'000 SNPs (for the purpose of this report we refer to it as chicken HDSNP array). Its development was based on extensive re-sequencing of a considerable number of chicken breeds (Kranis *et al.*, 2013). The combination of this new tool for genomic studies with a set of highly diverse chicken populations opens new possibilities to assess genomic architecture and distribution of genetic diversity at the molecular level.

Within the framework of the SYNBREED project, a project supported by the German Federal Ministry of Education and Research (www.synbreed.tum.de), a large panel of ~ 3000 individuals of more than 160 diverse chicken breeds has been collected. These chicken breeds were sampled in more than 25 countries and represent a wide phenotypic as well as genetic diversity within the species. For most populations phenotypic information on qualitative traits as skin color and comb shape is available, and for more than half of them individual body measurement have been collected, in addition to DNA samples. A standardized protocol for phenotypic characterization of genetic diversity in chickens has been developed for both discrete and continuous morphological traits. Since individuals were genotyped with the chicken HDSNP array, genome-wide high density SNP marker information is available. Hence, this set of populations collected within the framework of the SYNBREED project, which we call the "Synbreed Chicken Diversity Panel (SCDP)", is a vital and valuable resource for high resolution population genomic analyses in chickens.

The aim of this report is to introduce first results of these on-going research activities in a subset of 82 chicken breeds to illustrate the enormous diversity and potential of the SCDP.

Material and Methods

Sample collection

Chicken populations included in this report are a subset of the SCDP. Table 1 provides an overview about chicken breeds. The majority of the samples used in this study were collected from chicken fancy breeds in Germany between 2010 and 2012. The German Association of Poultry Breeders (Bund Deutscher Rassgeflügelzüchter e.V., BDRG) maintains a wide spectrum of traditional and fancy poultry breeds. They reflect various types of breeds supposedly originating from various regions according to the German Poultry Standard of Perfection (BDRG 2010, Table 1). Additional samples were collected from chicken breeds kept by farmers organized in The Society for the Conservation of Old and Endangered Livestock Breeds (GEH). Finally, the collection was completed by samples of two Red Jungle

fowl populations, *Gallus gallus gallus* and *Gallus gallus spadiceus*, as well as samples of commercial purebred chicken lines which were taken from previous EC project AVIANDIV (w3.tzv.fal.de/aviandiv/) and other subprojects of the SYNBREED project, respectively.

Blood sample were collected from wing vein of each bird using EDTA as anticoagulant. Sampling was carried out in strict accordance to the German Animal Welfare regulations, and noticed to the authorities of Lower Saxony according to § 8 of the German Animal Welfare Act. Breeders were asked for their agreement. In addition, several body measurements were recorded from the same individuals. These measurements included wing length (WL), shank length (SL), shank thickness (ST), and keel length (KL).

Table 1: Categories of chicken breeds used in this study (≥ 15 individuals per breed)

Origin	Type	Breeds *	# breeds
Asia	Long tailed breeds	*PHxx, SAsch, YOwr*	3
	Game type and related breeds	*ASrb, IKxx, Maxx, OFrbx, SHsch*	5
	Asian type breeds	*BHrg, BHwsch, COsch, DLIa, MRschk, NHbr, NHL68, ORge, PRgp, ROro, SNwsch, TOgh, WYsschs, WYw*	14
	Crested breeds	*SEsch, Sew*	2
Europe	Intermediate type breeds	*ARsch, ARwi, DOxx, VWco, VWcoE*	5
	Mediterranean type breeds	*ITrh, ITsch, KAsch, LER11, LEw, Misch*	6
	Northwest-European breeds	*AKxx, BKschg, BLxx, BSsch, DSgp, FRgew, HAsI, KRsch, KRw, LAco, OMsschg, RHrh, RHsch, THsch, WTs*	15
	Crested breeds	*APsscht, HOxx, PAxx*	3
Bantam	Asian type breeds	*CHgesch, CHschw, KSgw, OHgh, OHsh, ZCsch, ZCw*	7
	European type breeds	*ABwa, BAsch, DZgh, FZgpo, FZsch, GBxx, SBgschs, SBsschs*	8
Wild	Gallus gallus gallus	*GGg*	1
	Gallus gallus spadiceus	*GGsc*	1
Commercial lines	Broilers	*BRD_A, BRD_B, BRS_A, BRS_B*	4
	White Layer	*WL_A, WL_B, WL_C, WL_D*	4
	Brown Layer	*BL_A, BL_B, BL_C, BL_D*	4

* *Chicken breed names*

ABwa - Barbue d'Anvers quail

AKxx - Carlise Old English Game any colour

APsscht - Appenzeller Pointed Hood silver spangled

ARsch - Rumpless Araucana black

ARwi - Rumpless Araucana black breasted red

ASrb - Aseel red mottled

BAsch - Rosecomb Bantam black

BHrg - Brahma gold

BHwsch - Brahma light

BKschg - Bergische Crower

BLxx - Brakel silver

BSsch - Berg-Schlotter black

CHgesch - Japanese Bantam black tailed buff

CHschw - Japanese Bantam black mottled

COsch - Cochin black

DLIa - German Faverolles salmon

DOxx - Dorking any colour

DSgp - German Grey Chickens cuckoo

DZgh - German Bantam gold partridge

FRgew - Frisian Fowl chamois penciled

FZgpo - Booted Bantam millefleur

FZsch - Booted Bantam black

GBxx - Barbue du Grubbe any colour

KAsch - Castilians black

KRsch - Creeper black

KRw - Creeper white

KSgw - Ko Shamo black-red

LAco - Lakenvelder black and white

LER11- White Leghorn line R11

LEw - White Leghorn

Maxx - Malay black red

Misch - Minorca black

MRschk - Marans copper black

NHbr - New Hampshire red

NHL68 - New Hampshire line 68

OFrbx - Orloff red spangled

OHgh - Ohiki red duckwing

OHsh - Ohiki silver duckwing

OMsschg - East Friesian Gulls silver pencilled

ORge - Orpington buff

PAxx - Poland any colour

PHxx - Phoenix golden or golden duckwing

PRgp - Plymouth Rocks barred

RHrh - Rhinelander Chicken brown

RHsch - Rhinelander Chicken black

ROro - Rhode Island Red red

SAsch - Sumatra black

SBgschs - Sebright Bantam

SHsch - Shamo black

SNwsch - Sundheimer light

THsch - Thuringian Bearded Chicken black

TOgh - Toutenkou black breasted red

VWco - Vorwerk buff columbian

VWcoE - Vorwerk conservation program

WTs - Westphalian Chicken silver

WYsschs - Wyandotte silver laced

WYw - Wyandotte white

YOwr - Yokohama red saddled white

ZCsch - Pekin Bantam black

ZCw - Pekin Bantam white

GGg - Gallus Gallus Gallus

GGsc - Gallus Gallus Spadiceus

BRD_A - Broilers_A

BRD_B - Broilers_B

BRS_A - Broilers_C

BRS_B - Broilers_D

WL_A - White Leghorn line A

WL_B - White Leghorn line B

WL_C - White Leghorn line_C

HAsl - Hamburgh silver spangled	golden	WL_D - White Leghorn line_D
HOxx - Poland White Crested black	SBsschs - Sebright Bantam silver	BL_A - Rhode Island Red line A
IKxx - Indian Game dark	SEsch - Silkies black	BL_B - Rhode Island Red line B
ITrh - Leghorn brown	SEw - Silkies white	BL_C - White Rock line C
ITsch - Leghorn black		BL_D - White Rock line D

Genotyping and Filtering of SNP data

DNA Isolation from blood samples was done using standard phenol-chloroform method. Within the SYNBREED project, SNP genotyping was carried out at the Chair of Animal Breeding, Technische Universität München, using the chicken HDSNP array. This array comprises a total of 580'961 SNPs covering 28 autosomal chromosomes, two sex chromosomes Z and W as well as two linkage groups (LGE64, LGE 22C19W28). Annotation was done using Affymetrix 'Axiom_GW_GT_Chicken.na33.annot.csv' file. For the purpose of this study, SNPs located on Z and W chromosomes as well as on the two linkage groups were excluded. Filtering was done with 99 % SNP call rate, and an animal call rate of 95 %. After filtering, 445'264 SNPs and 1677 individuals of 82 populations, with 15 to 20 individuals per population, remained in the data set.

Selection SNP markers

Since selection of SNPs on the chicken HDSNP array was based on polymorphic sites discovered in sequences of a limited number of breeds, mainly of commercial origin, diversity in the wide spectrum of other breeds as studied here might be underestimated. This 'ascertainment bias' has been a matter of discussion for a long time (e.g. Albrechtsen *et al.* 2010). In order to reduce this disadvantage we selected 311'006 SNP loci for further analyses which have been found to be polymorphic in the two Red Jungle fowl populations. The reduced set of SNP loci represents about 55% of the SNP loci of the chicken HDSNP array. Across chromosomes, the reduction of SNP marker loci was almost equally distributed, except for chromosome 16 where chicken MHC is located (Figure 1). It is worth mentioning that SNP loci on the chicken HDSNP array are more uniformly distributed according to genetic distance than to physical distance on the final panel which is reflected in an increased marker density per 1 Mb on microchromosomes (Kranis et al., 2013).

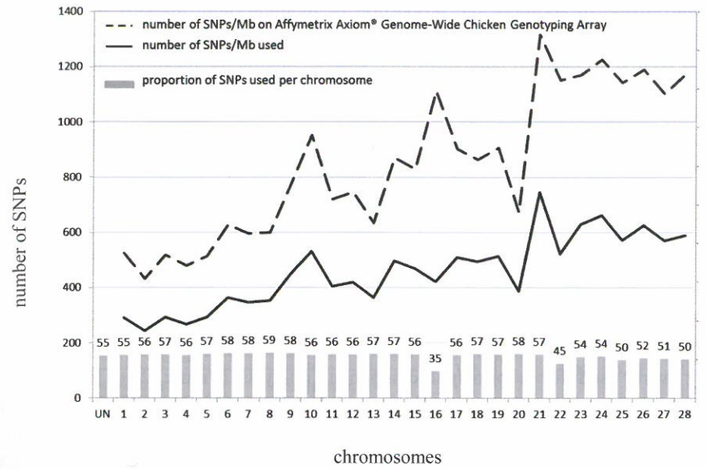


Figure 1: Chromosome-wise density of SNP of the chicken HDSNP array (*Affymetrix Axiom® Genome-Wide Chicken Genotyping Array*; dashed line) and of the reduced SNP panel (solid line) used in this study. Gray bars indicate the proportion of loci of the reduced SNP panel per chromosome compared to the full set of loci of HDSNP array (see text for more details)

Statistical analyses

Analyses were applied to molecular and phenotypic data separately. Calculations described below were based on *ad hoc* scripts written in R software package (R Core Team, 2013) if not indicated otherwise. In order to assess diversity within populations using SNP markers, the proportion of observed (H_o) and expected (H_e) heterozygotes per population were estimated per locus, and averaged across all chromosomes. Based on H_o and H_e estimates, F_{is} coefficient was calculated per population according to the concept of F-statistics developed by Wright (Wright, 1950). For both phenotypic traits and SNP markers, a Principal Component Analysis (PCA) was carried out. While for analyzing molecular data individual records were used, analysis of phenotypic data based on arithmetic means per population and trait. For phenotypic data, a scatter plot was drawn based on the first two principal components. For visualization of breed relationships based on molecular markers, Euclidean distances of the first five principal components were computed between all pairs of populations. This distance matrix was then converted to a Neighbor Joining tree using the Software package SPLITSTREE 4.0 (Huson and Bryant, 2006).

In addition, we searched for runs of homozygosity (ROH) which are stretches of individual's DNA in homozygote state. To achieve this, we used the algorithm implemented in PLINK software (Purcell *et al.*, 2007). The following criteria were applied to identify ROH in the individual's genome: the minimum length of a ROH was set to 300 kb with a minimum number of 100 SNPs. Maximum allowed gap size with no marker information within a run was 1000 kb. We allowed one heterozygote and one missing value per window to account for

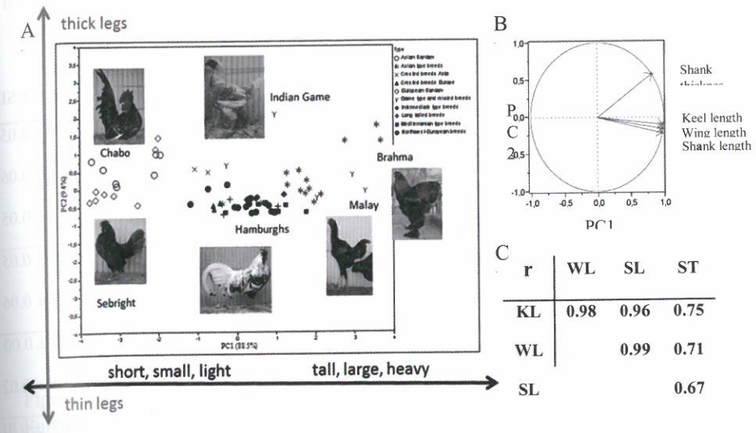
genotyping errors. In addition, the proportion of monomorphic markers was calculated by counting the number of SNPs in homozygote state per individual across the genome of an individual relative to the total number of loci, and calculating the mean for each population.

Results and Discussion

The chicken breeds analyzed in this report are a subset of the SCDP encompassing 82 populations. We selected this subset to study genetic diversity of diverse chicken populations collected in Germany in addition to two Red Jungle fowl populations as well as commercial purebred layer and broiler lines. Even though samples were collected in Germany only, the set of chicken breeds represented populations of various types and a wide distribution of origins and management.

Phenotypic traits

Population means of four body measurements WL, KL, SL and ST for 68 breeds sampled in Germany were used for a principal component analysis (Figure 2). The first principal component (PC1) explained 88.5% of the variation and differentiates populations mainly according to KL, WL and SL, while ST contributes to both PC1 and PC2. The body measurements KL, WL and SL were highly correlated, while ST showed lower correlation with other traits. Consequently, bantam breeds (e.g. Chabo and Sebright) with small body size were grouped together on one side of the spectrum while large breeds as Brahma and Malay can be found on the opposite side. Indian Game, also known as Cornish, and Brahma showed highest values of ST (16 mm to 18 mm) and clustered in the upper part of the plot. In addition to Sebright, a very small breed with extremely thin legs (5 mm), breeds of normal body size as Hamburgh or Lakenfelder showed lowest ST values (8 mm to 9 mm) and were placed in lower part of the plot. Overall, breeds of Asian background as for example Brahma, Deutsches Lachshuhn (similar to French breed Favorelles) and Sundheimer or Game birds as Malay are larger than Northwestern European breeds. Bantam breeds are clearly separated from normal sized chicken breeds, but no clear differentiation in body size related to Asian or European type of breeds was found.



r	WL	SL	ST
KL	0.98	0.96	0.75
WL		0.99	0.71
SL			0.67

Figure 2: Plot of first two principal components (PC) and their part of variation (%) for four body traits, Keel length (KL), wing length (WL), shank length (SL) and shank thickness (ST) (**A**); loading plot of the four variables (**B**); and correlation coefficients between four body

Diversity within chicken populations

Using the reduced marker panel of 311'006 SNPs, observed and expected degree of heterozygosity was calculated per population, and averaged across categories of populations according to their supposed origin (Table 2).

Table 2: Mean observed (H_o) and expected heterozygosity (H_e) estimates as well as fixation index (F_{is}) of different categories of chicken breeds across 311'006 SNP markers.

Region of Origin	Number of Populations	H_o Mean ± SD	H_e Mean ± SD	F_{is} Mean ± SD
Asia	24	0.23 ± 0.03	0.25 ± 0.03	0.07 ± 0.05
Europe	29	0.21 ± 0.04	0.23 ± 0.05	0.09 ± 0.06
Bantam	15	0.19 ± 0.05	0.20 ± 0.05	0.07 ± 0.05
European Bantam	*8*	*0.17 ± 0.06*	*0.18 ± 0.06*	*0.06 ± 0.05*
Asian Bantam	*7*	*0.21 ± 0.02*	*0.23 ± 0.03*	*0.07 ± 0.06*
Red Junglefowl	2	0.33 ± 0.01	0.34 ± 0.02	0.03 ± 0.00
Commercial	12	0.23 ± 0.07	0.22 ± 0.07	-0.06 ± 0.02
White Layers	*4*	*0.15 ± 0.01*	*0.14 ± 0.01*	*-0.07 ± 0.01*
Brown Layers	*4*	*0.23 ± 0.01*	*0.21 ± 0.01*	*-0.07 ± 0.01*
Broilers	*4*	*0.31 ± 0.03*	*0.31 ± 0.03*	*-0.03 ± 0.01*

The two Red Jungle fowl populations showed the highest degree of heterozygosity, which might be caused by the selection of SNP markers from the total panel of the chicken HDSNP array. On the other hand, the two populations are assumed to originate from wild ancestors that formed part of the founder populations of domesticated chickens. Between categories of chicken breeds, there are only small differences between mean estimates of heterozygosity. In general, chicken populations of Asian background tend to be slightly more diverse than European populations. It is noteworthy, however, that broiler purebred lines showed a high degree of variability compared to both commercial layer lines and chicken populations of other categories, while white layers displayed the least. Furthermore, commercial lines display a slight excess of heterozygotes compared to what is expected from allele frequencies assuming the population is in Hardy-Weinberg equilibrium (F_{is} estimates).

To get deeper insight into the degree of variability at the genome level, we searched for runs of homozygosity (ROH) across chromosomes. Figure 3 shows the association between the means per population of the total length of an individual's genome included in ROHs and the proportion of monomorphic markers. Both measures were highly correlated (r = 0.91).

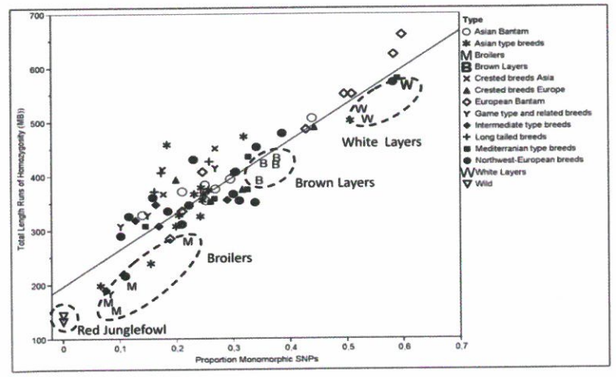


Figure 3: Mean proportion of monomorphic markers (X-axis) and mean total length of Runs of Homozygosity (Y-axis) per population.

In accordance to the selection procedure of SNP loci it is obvious that the two Red Jungle fowl populations do not show monomorphic markers. Furthermore, these two populations displayed the lowest proportion of the genome included in ROH. Compared to wild populations, the largest part of the genome included in ROH was found in the category of European Bantam breeds which also showed the highest proportion of monomorphic loci. Other populations showed a varying degree of homozygosity for both traits, the proportion of monomorphic markers and the proportion of genome stretches included in ROH. Regarding the commercial lines, the genome of white layer lines was least polymorphic. More than 50% of the 311'006 markers were monomorphic in these lines, and about half of the genome was included in ROH. This is in clear contrast to broiler lines, which clustered at the lower end of the spectrum, while brown layers showed a medium degree of variability. Assuming that ROH is a genomic measure of individual autozygosity (McQuillan et al., 2008), i.e. ROH are genome segments inherited from the same ancestor, either paternal or maternal, without occurrence of recombination or mutation (identical by descent), white layers displayed a much higher degree of inbreeding than brown layers, and even more than broilers. However, more careful analyses are underway to evaluate ROH considering the length of these stretches in the genome as well as the distribution across chromosomes. Furthermore, high ROH estimates in white layers do not correspond with F_{is} values found in this study. One possible explanation might be that in spite of lower effective population size of white layers than brown layers as found in other studies (Qanbari et al., 2010), mating of close relatives in current breeding populations is avoided.

Genetic Relationships between chicken populations

Based on a principal component analysis using individual genotypes at 311'006 SNP loci, the first five PCs were used to generate a distance matrix between populations which was then converted into a Neighbor Joining tree (Figure 4). Although Euclidian distances are not based on phylogenetic assumptions, this simple measure reflects a clear grouping between

populations which is in accordance to our knowledge about breed histories. Clustering discovered obvious separation between European breeds and breeds of Asian background.

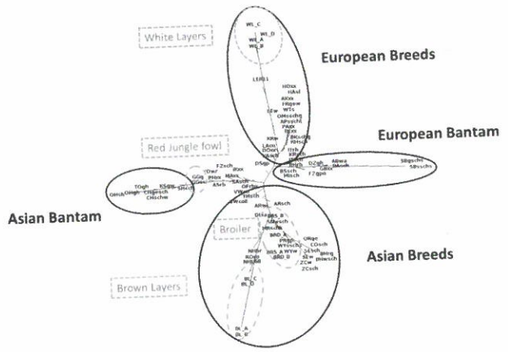


Figure 4: Neighbor Joining tree of 82 chicken populations based on Euclidian Distance calculated from first five Principal Components.

Although the introduction of chickens to Europe is still poorly understood, it has been assumed that they reached Europe by at least two routes more than 2000 years ago (West and Zhou, 1988). During the heyday of the Roman Empire, chickens spread from Mediterranean region to northern parts of Europe. It is likely, however, that chickens have already come earlier to Europe from Asia via Southeastern Europe. Up to the mid of the 19th century, chickens in Europe were of Bankiva type, and only afterwards Asian breeds as Cochin and Langshan from China became very attractive and found a wide distribution in Europe. They were maintained as pure breeds, but were also crossed into existing breeds in Europe forming a category of intermediate type breeds. The clustering presented in Figure 4 illustrates nicely the separation between European and Asian breeds, even though they were all collected in Germany. Furthermore, chicken breeds of European Bantam type are clearly distinguishable from those of Asian background. Both groups of autosomal dwarf chickens make up their own clusters distinct from chicken breeds of normal body size. The two Red Jungle fowl populations sampled in Thailand cluster closer to Asian Bantams than to others of the spectrum. Assuming that these two populations represent to some degree a part of the gene pool of founders, the distribution of chicken breeds studied indicated the wide variation accumulated during domestication. With respect to commercial purebred lines, white layers are clearly distinct from brown egg layers, while broiler lines cluster more in the center of the tree and closer to Asian than to European breeds. This, in turn, fits well to the history of these chicken lines. It is assumed that all White Leghorn lines used in commercial breeding of layers of white-shelled eggs originated from an Italian breed located in Tuscany (Livorno) in central Italy. The genetic basis of brown egg layers is broader than for white layers, utilizing Rhode Island Red, Plymouth Rock, Australorps, and New Hampshire among others, while broilers were based on mainly Cornish (Indian Game) and Plymouth Rock (Crawford, 1990).

Latter might also explain the closer relationship of broilers to brown layers than to white layers. Although white and brown layers have been selected for similar traits to increase laying performance, genetically they are little related to each other compared to the spectrum of chicken breeds studied. It is noteworthy that findings described above are in good agreement to earlier studies using microsatellites as molecular markers (reviewed by Groeneveld *et al.*, 2010).

Conclusions

This report gives first insights into the enormous diversity of the "Synbreed Chicken Biodiversity Panel" illustrating the value of this collection. So far, 1677 individuals of 82 populations mainly sampled in Germany have been included. In the framework of the SYNBREED project we managed to collect more samples covering regions outside Europe. Up to now, the SCDP encompasses about 160 chicken populations from 25 countries of Europe, Asia and Africa, and more than 3000 individuals were genotyped using the chicken HDSNP array. The high marker density generated in a this comprehensive and highly diverse gene pool, in combination with phenotypic traits, provide an extremely useful resource for high resolution analyses of phenotypic variability, architecture of the genome across divergent populations within the species, and for getting deeper inside into phylogenetic aspects of breed development. In first analyses using this huge data set, we focused on the assessment of the genetic variability within and the genetic relationships between populations under study. The usefulness of diverse chicken breeds to map genes or genomic regions associated to particular phenotypic traits has been demonstrated previously in other studies (e.g. Rubin *et al.*, 2010, Wragg *et al.*, 2012). A variety of promising other approaches is currently underway.

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L12 Welfare and breeding in broilers

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Abbreviated title: Welfare and breeding in broilers

Summary:

The demand for chicken meat continues to rise across the world, leading to calls for greater efficiency and sustainable intensification. This has implications for poultry welfare through potential conflicts between welfare and commercially valuable production traits such as rapid juvenile growth. However, selective breeding has the potential to positively improve chicken welfare alongside production traits by giving welfare higher priority in multi-trait breeding programmes. Multi-age breeding programmes that select for different growth rates in birds of different ages also has the potential to improve the welfare of parent birds (breeders), multi-age programme selection for different growth rates in different age birds.

Key words: welfare, growth rate, feed restriction, breeders, broilers

Introduction

A concern over how to feed the rising human population while at the same time minimizing the effect on the environment has led to calls for agriculture to become more 'sustainably intensive' and more efficient. (Royal Society, 2009; Steinfeld *et al*., 2006; Garnett *et al*., 2013; Gerber *et al.,* 2013). The human population is projected to be at least 9 billion by 2050 (FAOStat, 2009; Godfray *et al*., 2010) and the current trend is for the consumption of meat and dairy products to keep on rising (Gerber *et al*., 2013) as people become wealthier and want what they see as a better diet. Chickens are already, at 58 billion killed each year, the most commonly consumed animal (FAOStat, 2012) and projected to overtake pork by tonnage as well as numbers by 2020, with most of the increase is expected to occur in SE Asia and sub-Saharan Africa (USDA, 2013; Gerber *et al*., 2013).

Modern breeds of broilers are already highly efficient producers of protein due to a combination of diet, management and, in particular, selective breeding for high juvenile growth rate, breast meat yield and efficiency of food conversion (Flock *et al.,* 2005; Bessei, 2006; Estevez, 2007; Arnould and Leterrier, 2007). However, this selective breeding has had side-effects on the health and welfare of the birds including susceptibility to cardiovascular disease (Julian, 1995; Mitchell, 1997) and lameness (Kestin *et al.,* 1992; Rauw *et al*., 1998; Sanotra *et al.,* 2001; Bradshaw *et al.,* 2002; Burt, 2002; Knowles *et al.,* 2008). Selective breeding for fast juvenile growth rate has also had knock-on effects on the welfare of the parent birds ('breeders'). Without feed restriction, these breeder birds rapidly become obese (Dunnington and Siegel, 1985), have locomotory problems (Katanbaf *et al.,* 1989), the males have reduced fertility (McGary *et al.,* 2002). While these negative symptoms can be avoided by restricting the amount of food that the growing breeders receive, this is often only 25-50% of what the birds would consume if fed *ad libitum* (Savory *et al.,* 1993; Ducuypere *et al.,*