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Global diversity and genetic contributions of chicken populations from African, Asian and European regions

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Summary

Genetic diversity and population structure of 113 chicken populations from Africa, Asia and Europe were studied using 29 microsatellite markers. Among these, three populations of wild chickens and nine commercial purebreds were used as reference populations for comparison. Compared to commercial lines and chickens sampled from the European region, high mean numbers of alleles and a high degree of heterozygosity were found in Asian and African chickens as well as in Red Junglefowl. Population differentiation (F_{ST}) was higher among European breeds and commercial lines than among African, Asian and Red Junglefowl populations. Neighbour-Net genetic clustering and STRUCTURE analysis revealed two main groups of Asian and north-west European breeds, whereas African populations overlap with other breeds from Eastern Europe and the Mediterranean region. Broilers and brown egg layers were situated between the Asian and north-west European clusters. STRUCTURE analysis confirmed a lower degree of population stratification in African and Asian chickens than in European breeds. High genetic differentiation and low genetic contributions to global diversity have been observed for single European breeds. Populations with low genetic variability have also shown a low genetic contribution to a core set of diversity in attaining maximum genetic variation present from the total populations. This may indicate that conservation measures in Europe should pay special attention to preserving as many single chicken breeds as possible to maintain maximum genetic diversity given that higher genetic variations come from differentiation between breeds.

Keywords Marker-estimated kinship, core set analysis, genetic diversity, microsatellites, population structure

Introduction

Domestic chickens are thought to result from multiple domestication events over the last 8000 years (West & Zhou 1988; Sawai *et al.* 2010; Tixier-Boichard *et al.* 2011), predominantly of Red Junglefowl *(Gallus gallus)* in South-East Asia and, to a lesser extent, involving *Gallus sonneratii* in south-west India (Eriksson *et al.* 2008) and *Gallus lafayetii* in Sri Lanka (reviewed by Groeneveld *et al.* 2010; Tixier-Boichard *et al.* 2011). Later, the chicken spread to Europe and Africa through human migration and along trade routes (Liu *et al.* 2006; Kanginakudru *et al.* 2008; Groeneveld *et al.* 2010; Storey *et al.* 2012; Mwacharo *et al.* 2013a). The Iron Age (3000 B.C.) was the main period for dispersion of chickens through Europe. They came mainly from China via Russia on a northern route and from the Indus Valley via Persia on a southern route (West & Zhou 1988; Tixier-Boichard *et al.* 2011; Flink *et al.* 2014). The chickens were introduced to Africa from Asia by way of the Indian Ocean and from Europe and Arabian Peninsula via the Mediterranean and Red Sea (MacDonald & Edward 1993; Masonen 1995; Boivin *et al.* 2009). Archeological, linguistic and ethnographic evidence strongly suggests that the chicken moved to Africa in several waves from the Mediterranean region, Red Sea and the east coast of Africa with subsequent dispersion through overland routes across the Sahara, the Horn of Africa and central and West Africa (MacDonald & Edward 1993; MacDonald & Blench 2000; Williamson 2000). Gifford-Gonzalez & Hanotte (2011)

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reported two main waves of chicken introduction to Africa. The first wave was from the Mediterranean Sea via Egypt during the Ptolemaic period (300 B.C.), later spreading through the Nile Valley and to West Africa along the Sudano-Sahelian corridor (MacDonald & Edward 1993; Fuller *et al.* 2011). The second wave came across the Indian Ocean when chickens were introduced to the east coast of Africa by means of the existing trade networks during the beginning to middle of the 1st millennium A.D. (MacDonald 1992; Adelaar 1996; Blench 2006; Boivin & Fuller 2009; Fuller *et al.* 2011).

Genetic diversity within a species is defined by a large number of breeds and populations which exhibit a wide range of characteristics and variants. Genetic differentiation is expected to increase with both increasing geographic distance and demographic isolation (Wright 1943; Parker *et al.* 2004). The wide genetic variation between different breeds of domestic chickens which has been accumulated during domestication has several causes: founder effects at the time of domestication, subsequent isolation of breeds in different regions and under different environmental constraints that forced genetic drift and natural selection, selection imposed by man for breed standards and, most recently, selection for improving production traits. Genetic diversity is now distributed among traditional local chickens, standardized breeds selected according to a given breed standard and highly selected commercial lines (Tixier-Boichard *et al.* 2011). It has been claimed that the loss of the number of local populations, and hence a reduction in genetic variations due to the replacement of local chicken breeds in small farms and villages by modern industrial stocks, is driven by the increasing demand for animal products (Besbes *et al.* 2007; Halima *et al.* 2007; Boettcher *et al.* 2010; Hoffmann 2011; Özdemir *et al.* 2013). To counteract the loss of genetic diversity within the species, it is necessary to take conservation measures for animal genetic resources to retain the genetic potential of populations for flexibility in adapting to unpredictable future challenges. These conservation measures should be based on comprehensive insight and understanding of the importance of genetic diversity within the species (Weigend *et al.* 1995; Weigend & Romanov 2002; Rege & Gibson 2003; Simianer 2005; Oldenbroek, 2007; Lenstra *et al.* 2012). Adequate diversity analyses regarding the distinctiveness and demographic characterization of subpopulations are important when deciding conservation priorities (Groeneveld *et al.* 2010).

In this study, regional patterns of intra- and interpopulation genetic diversity of chicken populations from Africa, Asia and Europe were assessed using microsatellite variability. In this regard, the study took advantage of the availability of data from previous studies. The set of populations studied included 101 local populations from three continents as well as three Red Junglefowl populations and nine commercial pure breeds for comparison. A joint analysis of this comprehensive data set, which represents wide coverage in terms of geographical regions, climatic conditions and population management, allows a global assessment of diversity within the species based on diversity at 29 autosomal microsatellite loci. In African and Asian countries, chickens are kept mainly in free-range management systems (Abdelqader *et al.* 2007; Gondwe & Wollny 2007; Muchadeyi *et al.* 2007; Rajkumar *et al.* 2008), whereas non-commercial chicken breeds in Europe are of a smaller population size and bred for standardized traits (Granevitze *et al.* 2007; Siwek *et al.* 2013). By including populations with roots in two subspecies of Red Junglefowl, *Gallus gallus gallus* and *Gallus gallus spadiceus*, the study provides further insight into the degree of differentiation of domesticated chicken populations from their wild progenitors since domestication. The study aimed at evaluating genetic relationships within and between the 113 chicken populations included as well as assessing population stratification across regions of Africa, Asia and Europe. Then, these populations were compared to wild populations on the one hand and commercial lines intensively selected for egg or meat production and managed as isolated breeding populations for many selection generations on the other hand. Differences in the contribution of chicken breeds of different geographical regions to global diversity were investigated using multilocus microsatellite genotypes.

Materials and methods

Chicken populations

Data used in this study were collected from earlier studies (Muchadeyi *et al.* 2007; Bodzsar *et al.* 2009; Granevitze *et al.* 2009; Cuc *et al.* 2010; Mtileni *et al.* 2011a; Berima *et al.* 2013; Lyimo *et al.* 2013). Chicken populations included in this study encompassed various categories of management ranging from unmanaged backyard chickens to highly specialized commercial purebred chicken lines, sampled in various regions of Africa, Asia and Europe. All samples were genotyped at the same 29 microsatellite loci using the same laboratory protocol and standard samples to adjust for allele scoring between analyses. Twenty-eight of the 29 loci were taken from the list recommended by the ISAG/FAO advisory group for chicken biodiversity (FAO 2011).

Details of the sampling regions of breeds studied are shown in Table S1. In brief, chicken populations were categorized following different criteria. First, they were grouped in accordance with their breed history. The main geographical regions breeds originated from were sorted by continent. Within the continent, chicken populations were grouped into geographical subregions according to their breed history. The UN Geoscheme map based on M49 coding classification, which divides the world into macro-geographic regions and subregions (UNDATA 2012), was used to classify subregions. For Africa, chicken populations were grouped into three subregions: North Africa, East Africa and South Africa. In Asia, chicken populations were divided into the two subregions of East Asia and South-East Asia. The chicken populations sampled in Europe were classified into five groups: north-western European, eastern European, Mediterranean, a fourth group comprising a few breeds sampled in Germany that are of recent Asian origin, and a fifth group comprising a few populations related to brown layer as New Hampshire and Rhode Island Red chickens. Second, grouping was carried out according to sampling countries to represent the existing variations within geographic regions. Third, grouping was based on information on population management, that is field populations without population management, breeds selected for breed standard, conservation flocks and populations selected for quantitative performance traits.

A total of 3314 individuals from 113 chicken populations were included in this study. To limit over-representation of a breed, a maximum of 30 individuals per population was allowed. In cases for which data from more than 30 individuals per breed were available, the number was reduced randomly using the Research Randomizer tool (http://www.randomizer.org/form.htm). The samples represented 21 countries in Africa, Asia and Europe including 22 ecotypes from Africa, 26 breeds from Asia and 53 breeds from Europe. In addition, three wild chicken populations and nine commercial layer and broiler purebred chicken lines were added to the study. Red Junglefowl and commercial purebreds were used as reference populations in the analyses.

Statistical analyses

The presence of null alleles for each locus was estimated by the expectation-maximization (EM) algorithm of Dempster *et al.* (1977) using FREENA software (Chapuis & Estoup 2007). The observed allele frequencies and allele frequencies estimated based on the EM algorithm for each locus across populations were highly correlated (>0.98), and the estimated frequencies of null alleles per locus were below 5% (Table S2). According to Leroy *et al.* (2012) and Pham *et al.* (2013), frequencies of null alleles of below 20% are negligible. The percentage of the missing values of the microsatellite data was 0.63%. Allele frequencies, mean number of alleles (MNA), expected (H_E) and observed (H_O) heterozygosity of the populations were estimated using the MICROSATELLITE-TOOLKIT (Park 2001). Wright's fixation indices (F_{IS} and F_{ST}) were estimated using FSTAT 2.9.3.2 software (Goudet 2002).

Cluster analysis

The population structure was investigated using a model-based clustering approach as implemented in STRUCTURE 2.3.3 software (Pritchard *et al.* 2000; Falush *et al.* 2007; Hubisz *et al.* 2009). The analysis involved an admixture model with correlated allele frequencies. The length of the burn-in period was set to 50 000 iterations followed by 100 000 iterations for Markov chain Monte Carlo sampling. The user-defined number of clusters ranged from $2 \le K \le 40$. Individuals were grouped into a predefined number of clusters with 100 independent runs for each K value. The G' similarity coefficient based on the large K-greedy algorithm as implemented in CLUMPP software (Jakobsson & Rosenberg 2007) was used to compare STRUCTURE runs within each K value. Solutions with a similarity higher than 95% were considered to be identical (Granevitze *et al.* 2009). The most frequent solution was considered to be the most probable clustering, and a merger of these runs within each of the K values obtained from CLUMPP software was visualized using DISTRUCT 1.1 software (Rosenberg 2004). In addition, ΔK statistics suggested by Evanno *et al.* (2005) was applied to detect the number of clusters best reflecting the population structure (Fig. S2). For the most likely clustering solution ($K = 3$), distributions of membership coefficients obtained from STRUCTURE were subdivided into subregions, according to breeds' historic geographical origin, and displayed as pie charts (Fig. 2).

Similarity indices between and within populations were calculated from allele frequencies using Malecot's definition of similarity (Eding & Meuwissen 2001). These indices were subsequently used to calculate marker-estimated kinship (MEK) among populations using weighted equal drift similarity (weds) to correct for alleles identical by state (Oliehoek *et al.* 2006), which were executed in the MEKSAFE 1.0 software package (Eding *et al.* 2002). Mean kinship estimates within and between populations were obtained by averaging the corresponding values for all the within- and between-population pairs of individuals. The MEK estimates were converted to distances between populations (Eding *et al.* 2002; Mateus *et al.* 2004). The kinship-based distance matrixes among populations were visualized in a Neighbour-Net using SPLITSTREE4 software (Huson & Bryant 2006).

The genetic contributions of different chicken populations from each region to the total diversity were estimated according to Eding *et al.* (2002). Eding *et al.* (2002) proposed a core set analysis method, which is based on kinship estimates. This method accounts for both within- and between-population diversity instantaneously. The genetic variation contribution of each breed was estimated with minimum overlap of the core set, and ranking of populations was achieved according to their contributions to the global diversity. In addition, total genetic diversity of the nine commercial breeds was set as fixed and the additional contributions of the local populations to the commercial gene pool were computed by adding breeds one by one to the fixed set (Eding *et al.* 2002).

Results

Genetic diversity across regions

The mean number of alleles per locus and population was higher in African (MNA = 5.20 ± 0.17) and Asian chickens (MNA = 5.12 ± 0.16) than in European breeds (3.20 ± 0.11) and commercial lines (MNA = 3.28 ± 0.26). The mean number of alleles (MNA = 4.76 ± 0.038) and estimates of expected heterozygosity (MNA = 0.610 ± 0.035) found in Red Junglefowl were comparable to African and Asian chicken populations (Table 1). Higher estimates of expected heterozygosity were calculated for African ($H_E = 0.604 \pm 0.016$) and Asian ($H_E = 0.603 \pm 0.015$) chickens compared to European ($H_E = 0.455 \pm 0.011$) and commercial ($H_E = 0.453 \pm 0.026$) breeds. Moreover, European breeds showed a wider variation of heterozygosity across populations than did African and Asian chickens (Fig. S1). The breeds from eastern Europe ($H_E = 0.525 \pm 0.019$), the Mediterranean region ($H_E = 0.431 \pm 0.018$) and the group of breeds of Asian origins ($H_E = 0.489 \pm 0.028$) sampled in Europe displayed expected heterozygosity values which overlapped with Asian and African chicken populations at their upper end. The lowest estimates of European breeds were found in the north-west subgroup, which overlaps with commercial white layers at the lower part. African and Asian populations showed lower genetic differentiation ($F_{ST} = 0.108 \pm 0.004$ and 0.120 ± 0.005 respectively) compared to European and commercial breeds ($F_{ST} = 0.301 \pm 0.007$ and 0.327 ± 0.022 respectively). Among the European chickens, the north-west European breeds displayed, on average, the lowest expected heterozygosity ($H_E = 0.425 \pm 0.011$) within and highest F_{ST} (0.315 ± 0.010) value between pairs of populations, respectively (Table 1).

Population stratifications

Population stratifications of all 113 chicken populations were evaluated. A model-based clustering algorithm implemented in STRUCTURE and pairwise MEK distances visualized in Neighbour-Net were used to assess the population structure of the total pool of chicken breeds at various levels (Fig. 1). In the STRUCTURE analysis, at both $K = 2$ and $K = 3$, all pairwise comparisons of runs showed a G' similarity coefficient of >95% and were considered as identical. Likewise, $K = 3$ was the most likely number of genetic clusters as inferred by the method described in Evanno *et al.* (2005). Clustering found at these levels of resolution ($K = 2$ and $K = 3$) is comparable to the distribution of chicken breeds found in the phylogenetic tree. Both STRUCTURE and the analyses of kinship distances revealed two main clusters of Asian and north-west European chickens at opposite ends. African populations clustered between these

Table 1 Global genetic diversity distribution among a wide range of chicken populations

Sampling regions	Population (*n*)	$H_E \pm$ std	$H_O \pm$ std	MNA ± std	$F_{ST} \pm$ std	$F_{IS} \pm$ std
African chickens						
East Africa	6	0.625 ± 0.026^{a}	0.613 ± 0.026^{a}	5.66 ± 0.27^{a}	0.053 ± 0.005^{f}	0.019 ± 0.027^{a}
South Africa	12	0.624 ± 0.018^{a}	0.590 ± 0.018^{a}	5.23 ± 0.19^{ab}	0.075 ± 0.006^{f}	0.052 ± 0.019^{a}
North Africa	5	0.527 ± 0.028^{ab}	0.490 ± 0.029^{abc}	4.57 ± 0.30^{b}	0.157 ± 0.017^{de}	0.070 ± 0.029^{a}
	23	$0.604 \pm 0.016^{(1)}$	$0.574 \pm 0.018^{(1)}$	$5.20 \pm 0.17^{(1)}$	$0.108 \pm 0.004^{(3)}$	$0.047 \pm 0.015^{(1)}$
Asian chickens						
South-East Asia	16	0.640 ± 0.019^{a}	0.600 ± 0.020^{a}	6.04 ± 0.21^{a}	0.053 ± 0.006^{f}	0.063 ± 0.211^{a}
East Asia	10	0.590 ± 0.016^{ab}	0.563 ± 0.016^{a}	4.55 ± 0.17^{b}	0.142 ± 0.007^{de}	0.028 ± 0.016^{a}
	26	$0.603 \pm 0.015^{(1)}$	$0.576 \pm 0.017^{(1)}$	$5.12 \pm 0.16^{(1)}$	$0.120 \pm 0.005^{(23)}$	$0.042 \pm 0.014^{(1)}$
European chickens						
East Europe	12	0.521 ± 0.018^{ab}	0.510 ± 0.017^{ab}	3.45 ± 0.19^{d}	0.226 ± 0.011^{c}	0.021 ± 0.019^{a}
Asian background	5	0.489 ± 0.028^{bc}	0.444 ± 0.027^{bcd}	3.40 ± 0.30^{d}	0.282 ± 0.019^{bc}	0.091 ± 0.029^{a}
Related brown layer	4	0.474 ± 0.035^{bc}	0.442 ± 0.037^{bcd}	3.09 ± 0.19^{d}	0.258 ± 0.035^{bc}	0.066 ± 0.038^{a}
Mediterranean	12	0.431 ± 0.018^{cd}	0.400 ± 0.019^{cd}	3.18 ± 0.15^{de}	0.291 ± 0.016^{bc}	0.081 ± 0.029^{a}
North-west Europe	19	0.425 ± 0.011^{d}	0.360 ± 0.015^{d}	2.97 ± 0.15^{de}	0.315 ± 0.010^{b}	-0.133 ± 0.015^{a}
	52	$0.455 \pm 0.011^{(2)}$	$0.419 \pm 0.012^{(2)}$	$3.20 \pm 0.11^{(2)}$	$0.301 \pm 0.007^{(1)}$	$0.084 \pm 0.009^{(1)}$
Commercial lines						
Broilers	4	0.563 ± 0.031^{ab}	0.550 ± 0.032^{ab}	3.98 ± 0.33^{cd}	0.119 ± 0.017^{e}	0.024 ± 0.032^{a}
Brown layers	3	0.426 ± 0.035^{cd}	0.414 ± 0.037^{c}	3.02 ± 0.38^{d}	0.177 ± 0.047^{d}	0.027 ± 0.038^{a}
White layers	2	0.275 ± 0.043^{d}	0.264 ± 0.045^{d}	2.26 ± 0.47^{e}	0.470 ± 0.057^{a}	0.026 ± 0.047^{a}
	9	$0.453 \pm 0.026^{(2)}$	$0.441 \pm 0.028^{(2)}$	$3.28 \pm 0.26^{(2)}$	$0.327 \pm 0.022^{(1)}$	$0.026 \pm 0.023^{(1)}$
Red Junglefowl	3	$0.610 \pm 0.035^{ab(1)}$	$0.581 \pm 0.037^{ab(1)}$	$4.76 \pm 0.38^{ab(1)}$	$0.175 \pm 0.014^{d(2)}$	$0.046 \pm 0.038^{a(1)}$

Different superscript letters or numbers in a column indicate significant differences (Tukey's HSD, $P < 0.05$); different F_{IS} estimates were not significantly different from zero at $P > 0.05$.

H_E = expected heterozygosity, H_O = observed heterozygosity, MNA = mean number of alleles; F_{IS} = average inbreeding coefficient within subpopulation, F_{ST} = differentiation between subpopulations.

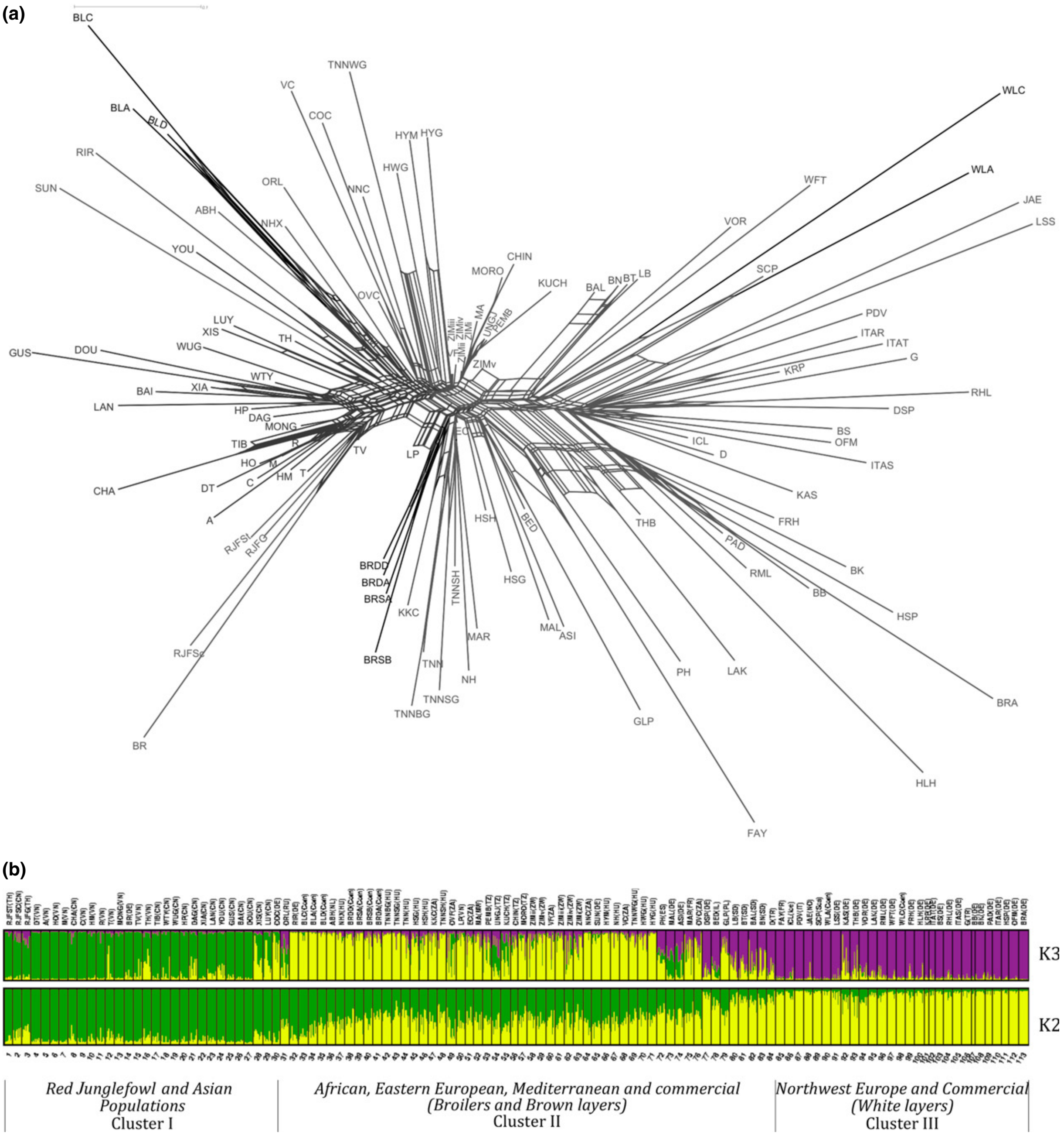

Figure 1 (a) Phylogenetic network tree of the chicken populations derived from marker-estimated kinship of 113 chicken populations from various origins; (b) population structure at $K = 2$ and $K = 3$ of 113 chicken populations from various origins (descriptions of the different breeds may be found in Table S1).

two clusters together with breeds sampled from eastern Europe, the Mediterranean region, populations related to the brown layer, broilers (BRDA, BRDD, BRSA and BRSB) and brown egg layers (BLA, BLC and BLD). However, chicken breeds sampled in Europe but originated recently from Asia clustered together with Asian chickens and Red Junglefowl (RJFG, RJFSC and RJFST). Within Asian chicken populations, Red Junglefowl had smaller genetic distances compared to chicken populations from South-East Asia than compared to East Asian populations.

Clustering of commercial chicken lines revealed obvious differences between white layers and brown layers, which both formed edges of the total spectrum of chicken populations at opposite sides. In contrast, the group of diverse broiler lines was found more in a central position of the spectrum. White egg layers (WLA and WLC) clustered with north-west European chickens. More specifically, white egg layers clustered together with other White Leghorn [i.e., line sarcoma susceptible (LSS) and the Scandinavian reference population (SCP)] as well as with Jaerhoens (JAE) and Padovana (PDV). The fancy breeds sampled in Germany [Italiener rebhuhnfarbig (ITAR), Italiener schwarz (ITAS), Italiener Triesdorf (ITAT), Kastilianer (KAS) and Pardoner (PAD)] which originated from Italy, clustered close to commercial white egg layers and other White Leghorn breeds. An Egyptian origin breed Fayoumi (FAY), which was sampled in France, clustered closely with the Mediterranean chickens Prat (PH) from Spain and Bedouin (BED) from Israel. Commercial brown egg layers clustered with Rhode Island Red (RIR); AB_High line (ABH), which is based on an Isa Warren cross; Gödöllö NHX (NHX), a New Hampshire cross maintained as a conservation flock in Hungary; and the Orlov (ORL) chicken breed. Vietnamese breeds [Te (T), H'mong (HM), Ac (A), Choi (C), Mai (M), Dong Tao (DT), Ho (HO)] clustered closely to Red Junglefowl and Brahma (BR) chickens, a breed of Asian origin but sampled in Germany. In addition, Sundheimer (SUN), a breed sampled in Germany with influences of populations from Asia, was grouped in the East Asian main cluster. Chickens of South African conservation flocks of the Koekoek (KKC), Venda (VC), Naked Neck (NNC) and Ovambo (OVC) breeds clustered away from other African populations and shared a cluster with eastern European breeds and broiler chickens.

Means for the geographical subgroups of chicken populations (Table S3) were calculated from the individual membership coefficients of the most probable STRUCTURE clustering (i.e., $K = 3$). Red Junglefowl, East Asian and South-East Asian chicken populations showed very similar patterns with one cluster dominating in Asian breeds (Fig. 2). Breeds of Asian background sampled in Germany had a higher membership coefficient in the cluster of Asian chicken populations and represented a very low proportion in the north-west European cluster. The distribution of membership coefficients for commercial brown layers (BLY) was similar to non-commercial populations related to brown layers (RBL). Among African chickens, East African chickens had higher membership coefficients in the cluster dominated by Asian chicken breeds and South African chickens showed a higher membership proportion in the cluster encompassing East European breeds. North African breeds showed a high membership coefficient in the cluster dominated by chickens from the Mediterranean region and north-western Europe.

Genetic contributions

The contributions of the chicken populations across regions to an optimal core set of global diversity are shown in Table 2. Red Junglefowl populations provided the highest contribution (0.0191 ± 0.0016) to an optimal core set, followed by Asian chicken populations (0.0115 ± 0.0006) and broilers (0.0106 ± 0.0014). On average, the contribution to the core set of a single European population (0.0069 ± 0.0004) and white layers (0.0061 ± 0.0019) was lowest. The correlation between core set contributions and average kinship estimates was highly negative ($r = -0.962$; Fig. S3), implying that higher kinship levels between individuals within a population result in smaller genetic contribution to the core set.

Considering diversity of commercial lines as a fixed set, with exception of Deutsche Sperber (DSP) and Bergische Schlotterkaemme (BS), all populations showed a positive contribution to this set (Table S4). On average, European breeds contributed significantly less (0.1262 ± 0.014) to the commercial gene pool compared to African (0.3646 ± 0.021) and Asian (0.3571 ± 0.019) chicken populations (Table 2).

Discussion

Comprehensive knowledge of population stratification and the distribution of genetic variability in breeds and strains are important factors when considering conservation measures with the aim of maintaining sufficient genetic diversity within a species for future generations. In this study, the level of diversity of chicken populations from various continents and with differing population histories, their genetic relationships and the contributions of populations to global diversity were studied.

Genetic diversity across regions

Cluster analyses identified three main groups of populations. Phylogenetic network and STRUCTURE analysis showed clearly distinct gene pools of Asian and north-western European chicken populations. African, south-east European and brown layer breeds as well as broiler lines formed a third group between the Asian and north-western European chicken gene pool. In comparison with European breeds kept mainly by fancy breeders, African and Asian chickens exhibited a higher degree of heterozygosity and a higher mean number of alleles per locus and population. According to many reports, local chickens from Africa and Asia are characterized by extensive phenotypic variations within and between different populations (Msoffe *et al.* 2001; Muchadeyi *et al.* 2007; Mwacharo *et al.* 2007; Chen *et al.* 2008; Rajkumar *et al.* 2008; Cuc *et al.* 2010; Dana *et al.* 2010; Mtileni *et al.* 2011a; Leroy *et al.* 2012; Lyimo *et al.* 2013). The traditional management system still in use at the present is defined by the absence of selection practice and by uncontrolled breeding management. This may have contributed to higher variation within the populations of this type (Abdelqader *et al.* 2007; Gondwe & Wollny 2007; Rajkumar *et al.* 2008).

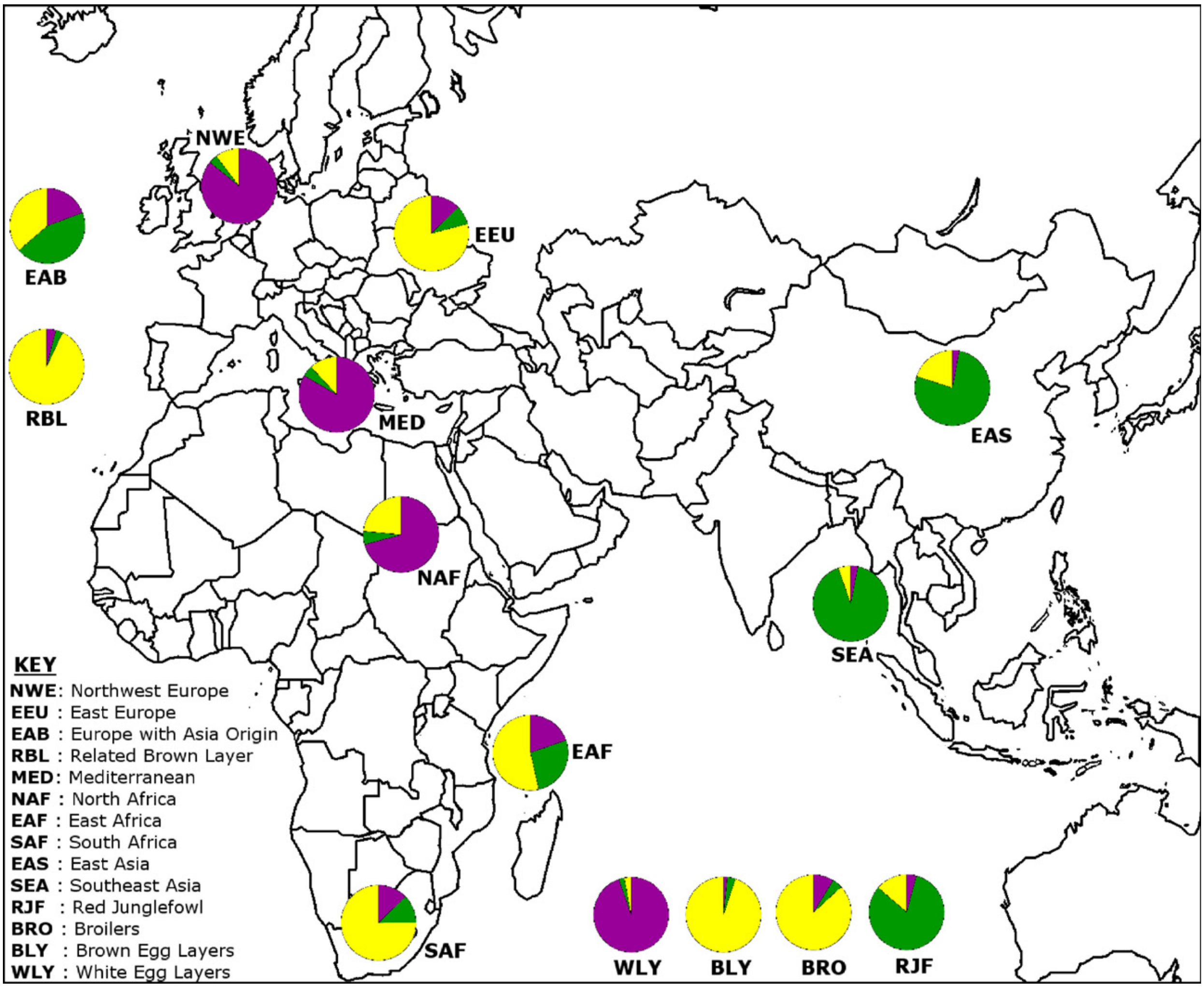

Figure 2 Map showing distributions of STRUCTURE mean membership coefficients of the most probable clustering at $K = 3$ of pools of chicken populations across various regions.

Table 2 Average contribution of various categories of chicken groups to the core set and additional contribution to the safe set when commercial lines were fixed as reference populations

Sampling region/ Chicken type	Optimal core set *c(i)*	Contribution to the safe set *c(i)′*
Red Junglefowl	0.0191 ± 0.0016^{a}	0.3877 ± 0.056^{a}
Asia	0.0115 ± 0.0006^{b}	0.3571 ± 0.019^{a}
Africa	0.0089 ± 0.0006^{c}	0.3646 ± 0.021^{a}
Europe	0.0069 ± 0.0004^{d}	0.1312 ± 0.014^{b}
Broilers	0.0106 ± 0.0014^{b}	–
Brown layers	0.0085 ± 0.0028^{c}	–
White layers	0.0061 ± 0.0019^{d}	–

Different superscript letters in a column indicate significant differences (Tukey's HSD, $P < 0.05$).
c(i), the relative contribution to the core set for breed *I; c(i)′* core set contribution of population *i* when added to the safe set.

Although European breeds displayed a lower average heterozygosity compared to African and Asian breeds, they exhibited a wider distribution of heterozygosity estimates across populations than did African and Asian chickens. This indicates a wider variation in diversity between European fancy populations, which is to some degree related to a varying degree of population sizes.

Asian gene pool

According to phylogenetic Neighbour-Net, Red Junglefowl clustered closely with South-East Asian chicken populations. The close association of South-East Asia chicken populations with Red Junglefowl was also reported in previous studies (Collias & Saichuae 1967; Berthouly *et al.* 2009; Cuc *et al.* 2010). Berthouly *et al.* (2009) showed an admixture of H'Mong chickens from northern Vietnam with wild jungle fowls, indicating gene flow from wild to domestic populations of South-East Asia. The chickens sampled in Europe with a recent Asian background (Brahma, Cochin and Sundheimer) were closely related to the Asian gene pool. Even though these populations have been kept in Europe for more than 150 years, either directly originating from Asian breeds or from crosses of local strains with Asian breeds, as in case of Sundheimer, they still retain their origins.

North-west European gene pool

The standardized breeds of chickens sampled from north-western Europe formed a gene pool distant from the Asian gene pool. This pool also encompasses commercial white egg layers, fancy breeds of Mediterranean origin (Italiener rebhuhnfarbig, Italiener schwarz, Italiener Triesdorf, Kastilianer and Paduaner) and other White Leghorn breeds (Line Sarcoma Susceptible and Scandinavian reference population). Icelandic Landrace 'Landnamshaena' is the native breed of Iceland, situated more than 2000 km away from the European mainland. Although isolated from others, this population clustered together with north-west European chicken populations. Several reports indicated that the Icelandic Landrace might have originated from the Old Norwegian Jadar and North German chicken breeds (Kirby & Hainkkanen 2000; Ball-Gisch 2009; Heinrichs 2010). Icelandic Landrace clusters closely with the old German breeds Bergische Schlotterkaemme, Ostfriesische Möwen and Italian Black sampled from German fancy breeders, although to the best of our knowledge no historic records are available explaining this immediate relationship. The Icelandic chickens were brought to the island between the 9th and 10th centuries by Norse settlers who established Viking settlements on Iceland (Grote 2006; Ball-Gisch 2009; Aviculture-Europe 2010).

African–south-east European gene pool

These groups of chicken populations clustered in the middle of the phylogenetic network between the Asian and north-west Europe gene pools. STRUCTURE analysis revealed an admixture of Asian and north-west European at $K = 2$ (Fig. 1b). The admixture of African, Mediterranean and south-east Europe chickens could be related to the geographical junction of Asia, South Europe and northern Africa through immediate interactions surrounding the Mediterranean Sea. Moreover, colonization and early human interactions through cross-regional trade routes and prehistoric migration trails could also contribute to this admixture. A map showing the distributions of STRUCTURE membership coefficients (at $K = 3$) indicated that North African chickens share more of their ancestral gene pool with European breeds than do Asian chicken populations (Fig. 2). This finding is in line with historical records of the introduction of chickens to Africa and is also confirmed by mitochondrial DNA analysis. Several mtDNA studies showed that chicken populations from North Africa are commonly described with haplotypes more frequent in Europe than those from chickens sampled in eastern Africa (Muchadeyi *et al.* 2008; Mwacharo *et al.* 2011, 2013b). Coltherd (1966) reported that the chicken arrived in Africa for the first time mainly from Europe through Egypt via land trade routes and/or sea-born trade across the Mediterranean Sea. Mwacharo *et al.* (2011) reported another link between North Africa and the Arabian Peninsula, which were connected by a terrestrial route.

Cluster analyses visualized as a Neighbour-Net tree derived from MEK distances and the STRUCTURE results indicate that South African breeds maintained as conservation flocks clustered closely with the European breeds and commercial purebreds. The Ovambo, Naked Neck and Venda South African conserved breeds were clustered closely with Hungarian breeds and brown layers, and the Koekoek conserved breed was clustered with broilers. This suggests that these chickens maintained as conservation flocks in South Africa may possess part of the genetic make-up of European breeds and commercial purebred lines. Mwacharo *et al.* (2013b) reported the arrival of European settlers to Africa in the 15th century A.D., opening the opportunities for the influx of European chickens to Africa. Van Marle-Köster *et al.* (2008) reported that chickens were introduced to southern Africa during the 1600s by early settlers and traders from Europe and sub-Saharan Africa. Black Australorp, White Leghorn and Plymouth Rock chickens were introduced to the former Potchefstroom Agricultural Research Institute during the late 1940s and used as parental stock for the Koekoek breed (Van Marle-Köster & Nel 2000). The clustering of Koekoek chickens with broiler chickens might be due to introgression of Plymouth Rock chickens, which were also used as a parental stock for purebred broilers. In contrast, South African chicken populations sampled from the field clustered together with East African scavenging chickens. Based on mtDNA studies, East African chickens have their roots to a large degree in South-East Asia and the Indian subcontinent (Muchadeyi *et al.* 2007; Mwacharo *et al.* 2011; Lyimo *et al.* 2013). Mtileni *et al.* (2011b) found the majority of South African village chickens also originated from the Indian subcontinent and South-East Asia.

Influence of Mediterranean chickens

Several reports confirmed the vast dispersal of Mediterranean chickens in different regions. This might be influenced by earlier (3000 B.C.) introductions of chickens from Asia (West & Zhou 1988; Tixier-Boichard *et al.* 2011), the geographical location of the Mediterranean region and the region being among the earliest centers for the historical cross-cultural and trade links (Curtin 1984; Sherratt & Sherratt 1993; Tracy 1993). West & Zhou (1988) provided archeological evidence of chickens' existence in Iran, Turkey, Syria, Greece, Romania and Ukraine at an earlier date, before the Mohenjo-Daro settlement (2600 B.C.). The Mediterranean region is crucial to understanding the origins and development of many modern societies, and it was an important route for merchants and travelers of ancient times that allowed for trade and cultural exchange between emergent peoples of the Mesopotamian, Egyptian, Phoenician, Carthaginian, Iberian, Greek, Macedonian, Illyrian, Thracian, Levantine, Gallic, Roman, Albanian, Armenian, Arabic, Berber, Jewish, Slavic and Turkish cultures (Ancient History Encyclopedia 2011). MEK and STRUCTURE analyses revealed that chickens of Mediterranean origin were widespread in Europe. Historical and archeological evidence suggests that indigenous chickens from the East and Central Europe might have dual origins with a northern diffusion from the Black Sea (Revay *et al.* 2010) and possible arrival from China through the Russia route (West & Zhou 1988; Tixier-Boichard *et al.* 2011).

Commercial lines and population related to brown layer

The differences between classes of commercial lines were clearly observed in the phylogenetic Neighbour-Net tree derived from MEK distances. The commercial lines of broilers and brown layers were clustered separately in the African–south-east European gene pool. Breeding of both broilers and brown layers was developed from Asian and Mediterranean chickens (Crawford 1990). The commercial broiler birds are crosses between Plymouth Rocks, Cornish Indian game bird, Langshans, Brahma and New Hampshire (Hathaway *et al.* 1953; Crawford 1990). Early attempts to produce hybrid chickens for meat were in the 1930s, and the intensification of the broiler industry started in the late 1950s (Gyles 1989; Griffin & Goddard 1994). Broiler chickens have been intensively selected for rapid growth rate, feed conversion efficiency and meat yield. Brown layer lines were developed in Rhode Island in the USA from a cross between Cochin, Red Malay game fowl, Brahma, Shanghai, Wyandotte and the Mediterranean breed Brown Leghorn. The Rhode Island Red breed was originally a dual-purpose fowl and admitted to the American Poultry Association standard of perfection in 1904 (Anderson 2013; Garrigus 2014). Since the 1940s, the Rhode Island Red has been selected for high egg production (Potts 2012). The populations related to brown layers (RIR, ABH, NH and NHX) clustered with commercial brown layers as they have their roots, to some degree, in the Rhode Island Red breed (AVIANDIV 2000).

Both STRUCTURE and phylogenetic clusters indicated that non-commercial White Leghorn chicken breeds (LSS, SCP) sampled in north-west Europe clustered closely with commercial white egg layers (WLA, WLC) which are based on the same breed (Crawford 1990). The White Leghorn breed originated from a native breed in central Italy (Livorno) and has been developed into commercial egg layers in United States since 1865. Today, a single comb White Leghorn is the most popular breed, leading in the production of white eggs worldwide (Global Poultry Trends 2013; Garrigus 2014). The majority of egg-laying breeds of chickens have ancestries that trace them to the Mediterranean class of chickens (Crawford 1990). Layer breeds have been genetically selected for high egg productivity.

Regarding population differentiation, European populations showed F_{ST} estimates very similar to those of commercial lines, which are kept as closed breeding populations with limited gene flow. This confirms that European chickens have been kept as isolated breeds with small effective population sizes, as reported previously (Granevitze *et al.* 2007). Populations with low genetic variability have a low genetic contribution to the core set. Although the majority of the European chicken breeds (96.23%) add genetic diversity to the commercial gene in a safe set analysis, their contributions are significant lower than that of Asia and African populations. Higher genetic differentiation and low genetic contributions have been observed in European breeds. Because the genetic variation is higher between European chicken populations, attention should be drawn to conservation of some European breeds in order to maintain maximum genetic diversity.

Conclusions

Merging data of several studies analyzed with the same set of molecular markers provided comprehensive insights into the genetic diversity of chickens across continents. Even though the number of loci is low compared to contemporary molecular tools, such as high-density SNP arrays or genome sequences, some general conclusions can be drawn from this study. Assuming that the two Red Junglefowl populations originated in a straight line from wild ancestors, which formed the founder gene pool of domestic chickens, phylogenetic analyses in this study illustrated that a wide variation has accumulated during domestication. Forces such as genetic drift as well as natural- and man-induced selection accompanied by isolation as a result of human migration have led to separated gene pools generally corresponding to geographical locations. The Asian gene pool, which is more closely related to wild ancestors, is more polymorphic than is the European gene pool. As reported for other species as well, this might be related to diversity decreasing when one moves outwards from the centers of domestication (Groeneveld *et al.* 2010; Wiener & Wilkinson 2011) but also to current breeding practices in European chicken breeds and the small size of these populations. As a consequence, a single European breed contributes only little to global diversity, but, in sum, they display a considerable part of the global diversity of the species. Furthermore, European chicken breeds which have been influenced by Asian breeds imported to Europe since the middle of the 19th century are still clearly distinguishable from European breeds originating from chickens introduced to Europe over 3000 years ago during the Iron Age (Northwestern European type). African breeds, introduced in several waves to the continent, make up a cluster originating from Asia but less distant than European breeds. Furthermore, close relationships to Europe may explain the influence of European breeds on North African and South African chicken breeds. In contrast to European breeds, these chicken populations have not been selected for breed standards, and management in free-range systems has maintained a high degree of variation with low differentiation between them.

Finally, commercial purebred layer lines made up outstanding clusters of the total spectrum separating white layers from brown layers. Although both types of lines have been selected for high laying performances, they are very distinct from each other according to their different breed origins, whereas broilers cluster more inside the spectrum of diversity. More importantly, these populations contribute only little global diversity, and replacing local populations by commercial lines will lead to tremendous reduction of diversity within the species.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 Distribution of heterozygosity among the regional chicken populations.

Figure S2 Estimation of most appropriate number of populations according to Evanno *et al.* (2005).

Figure S3 The correlation between marker estimate kinship and genetic contribution to the core set for 113 chicken populations.

Table S1 Source of data, sampling country, breed history, geographical distances from South-East Asia (SEA), core set contributions, genetic diversity between and average ($K = 3$) cluster membership coefficients (CMC) for 113 chicken populations.

Table S2 Estimated and observed allele frequencies, correlation coefficients and estimated null allele frequency based on EM algorithm (Dempster *et al.* 1977) for 29 microsatellite markers used in analyzing 113 chicken populations.

Table S3 Average membership coefficients for the regional and sub-regional chicken populations described by historical origin of the breeds.

Table S4 Diversity contributions of 104 chicken populations when commercial breed lines were considered safe from extinction.