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Genetic structure of a wide spectrum chicken gene pool

Z. Granevitze[1], **J. Hillel**[1,*], **M. Feldman**[2], **A. Six**[3], **H. Eding**[3], and **S. Weigend**[3]

[1]The Robert H. Smith Institute of Plant Sciences & Genetics, The Hebrew University of Jerusalem, Faculty of Agriculture, Food and Environmental Quality Sciences Rehovot 76100, ISRAEL

[2]Department of Biological Sciences, Stanford University, California, 94305, USA

[3] Institute of Farm Animal Genetics, Friedrich Loeffler Institute; 31535 Neustadt - Mariensee; Germany

Abstract

The genetic structure of 65 chicken populations was studied using 29 SSR loci. Six main clusters which corresponded to geographical origins and histories were identified: Brown Egg Layers, predominantly Broilers, native Chinese breeds or breeds with recent Asian origin, predominantly breeds of European derivation, a small cluster containing populations with No Common-History (NCH), and populations that had breeding history with *White Leghorn*. Another group of populations that shared their genome with several clusters was defined as “Multi-clusters”. *Gallus gallus gallus* (Multi-clusters), one of the subspecies of the Red Jungle Fowl, which was previously suggested to be one of the ancestors of the domesticated chicken, has almost no sharing with European and White Egg layer populations. In a further sub-clustering of the populations, discrimination between all the 65 populations was possible, and relations between them were suggested. The genetic variation between populations was found to account for about 34% of the total genetic variation, 11% between clusters and 23% between populations within clusters. The suggested clusters may assist in future studies on genetic aspects of the chicken gene pool.

Keywords

chicken; genetic diversity; *STRUCTURE*; biodiversity; SSR

Introduction

Traditional methods of clustering individuals and populations such as phylogenetic trees are based mainly on genetic distances. Recently, several studies have clustered individuals and populations of different organisms using *STRUCTURE* software, which uses Bayesian type clustering techniques (Pritchard *et al*. 2000, Rosenberg *et al.* 2001; 2002, Parker *et al.* 2004). Rosenberg *et al*. (2001) used genotypes of 27 SSR loci to assign 600 chickens originating in 20 populations to their original populations using *STRUCTURE* software; they reported on 98% success rate. They showed that when 12-15 highly polymorphic SSR loci and 15-20 individuals from each of 20 breeds were used, the clustering success was at least 90%. A gradual increase in the number of clusters in the analysis gives a perspective which can be interpreted as a reflection of the genetic relations between the populations (Hillel *et al.* 2007).

[*]Corresponding author - phone: +972-8-948-9957; Mobile: +972-54-882-0169; fax: +972-8-9489614; hillel@agri.huji.ac.il.

[#]Henceforth the population numbers are indicated in Figure 1 and Table 1.

The chicken is an important livestock species since it produces a large part of human food consumption and also being used as a model organism. In the recent era many chicken breeds and lines were diminished, with many breeds in danger of extinction (Blackburn 2006). This decline in the genetic resources of chicken around the globe may limit flexibility of future breeding and may jeopardize unique genetic features. Thus it is important to take steps to conserve genetic diversity within the species. For that purpose, deeper insight into genetic diversity and its stratification among chicken populations around the world is needed. In the current study we characterized the genetic diversity among 65 chicken populations from several geographical regions and with different breeding histories. Previously, biodiversity studies based on individual genotypes of chickens have been reported on phylogenic relationships among several local populations, commercial breeds or wild populations (Twito *et al*. 2007; Cuc *et al*. 2006; Chen *et al*. 2008, Muchadeyi *et al*. 2007). Unlike the current study, which is based on genotypes of individual birds, a biodiversity study on 52 populations, which partly overlaps with the set of the 65 populations used in the current study, has been reported by Hillel *et al.* (2003) using DNA pools of the populations.

In the current study we estimated the stratification of a wide range of chicken populations from several continents and management histories. This study adds the genetic relation among populations to our previous study on genetic diversity within these populations (Granevitze *et al*. 2007). Additionally, we suggest a procedure which makes it possible to use *STRUCTURE* on large datasets with a reasonable repeatability.

Materials and methods

Chicken populations

A list of the 65 chicken populations under study is shown in Table 1, and their detailed description is given in Granevitze *et al.* (2007). Briefly, the gene pool comprised one subspecies of the red junglefowl, *Gallus gallus gallus*, 12 native Chinese breeds, one population from Vietnam, one population from Malawi, 39 breeds of different phylogenetic origins collected in Europe (mainly in Germany), four brown egg layer lines, three of them being of commercial origin (A, C and D) and one experimental, two white egg layer lines, one commercial and one experimental, two broiler sire lines, and two broiler dam lines. An additional inbred line was used as a reference. We aimed to sample the same number of males and females from as many families as possible, for a total of approximately 30 chickens per population. In total, 2,000 individuals were analysed.

Genotyping

A detailed description of the 29 SSR (Simple Sequence Repeat) markers used in this study is given in Granevitze *et al.* (2007). Briefly, a set of 29 SSR markers which are distributed on 15 chromosomes of the chicken genome with a minimum distance of 17cM, were typed individually by means of PCR. Genotyping was performed using ABI sequencers (Applied Biosystems, CA, USA) and semi-automated LICOR sequencer (LICOR Biotechnology Division, Lincoln, Nebraska, USA).

Analyses, based on *STRUCTURE*

We used the *STRUCTURE* software (Pritchard *et al.*, 2000) to cluster the 2,000 chickens (from 65 populations) based on their genotypes at 29 SSR loci. In order to determine the number of iterations and burn-in periods needed for each solution, we created a curve of likelihood vs. number of iterations and burn-in steps (iterations and burn-in steps varied between 2,000 and 2.5 million steps; whole set at K=2). The curve reached saturation (plateau) at 10,000 iterations. We used 50,000 iterations after 20,000 burn-in cycles in all

the analyses. We analyzed the data for two to six clusters (K) with 100 repeats for each K value. Further analysis was done by splitting the dataset into subsets according to the clustering obtained at K = 6.

The similarity coefficient (C) measure, quantifies the similarity between two solutions of *STRUCTURE* calculated for the same number of populations with same number of clusters (Rosenberg *et al.*, 2002). We estimated similarity coefficients (C) between paired combinations among all 100 repeated solutions of *STRUCTURE* within each K-value. As was reported by Rosenberg *et al.* (2002) and seen in Figure 1, similarity coefficient (C) decreased rapidly for all K-values when similarities between solutions are slightly decreased. Pairs of solutions which had similarity coefficient above 0.95 presented similar clustering of populations with minor changes in membership coefficients; below this threshold clustering of populations were sometimes different. Thus, solutions with a pair-wise $C \geq 0.95$ were considered to be identical. The most frequent solution among the 100 repeats was chosen as the most likely. Graphical display of this solution was done using *DISTRUCT* software (Rosenberg *et al.* 2004). Due to computational limitations it was not possible to calculate the similarity coefficient (C) between the 100 runs for K values larger than six.

Phylogenic and statistical software

Analysis of molecular variance (AMOVA) was performed using *ARLEQUIN* (http://anthro.unige.ch/arlequin). The significance of the variance components associated with the different levels of genetic structure was tested using a non-parametric permutation procedure with 16,000 permutations (Excoffier *et al.* 1992).

Results and Discussion

Genetic structures of the 65 chicken populations were analyzed by the software *STRUCTURE*, applying an increasing number of K-values (from two to six) to the total set of 2000 individuals (Figure 2). At K = 2, the obtained two clusters separated the Asian and the European populations. A number of populations had average membership coefficients (graphically displayed as stripes) in both clusters indicating admixture of both. The cluster which corresponded to the Asian population included 12 native Chinese breeds, one Vietnamese population, and two German fancy breeds *Cochin*(#54) and *Brahma*(#55). The latter two breeds are German fancy breeds, which are presumably of Chinese origin (Wandelt and Wolters, 1996). The other cluster contained populations primarily originating from Europe. For K=3, the admixed populations from K = 2 formed a new cluster (in red at level K=3 in Figure 2). When K was increased up to six, the populations of this third group were further split into two separate main clusters, and a group which combined admixed populations of all six clusters (multi-cluster populations). One of these two main clusters, contained the *Asil*(#48) breed, broilers and a few additional related populations, which have partly an Asian background (Figure 2, red stripes). The second main cluster contained the brown egg layers based on *Rhode Island Red*(#41) and related populations (Figure 2, green stripes). At K = 5, a group of populations with No Common History (NCH) split off, encompassing breeds with no obvious relationship between each other (Figure 2, blue stripes). At K = 6, the White Egg Layers separated from the European cluster (Figure 2, gray stripes). *White egg layers* were established in the USA and were based on a narrow genetic basis of a single breed, the single comb *White Leghorn* (Crawford *et al*. 1990). The frequencies of the most frequent solutions are presented in quadrangular parentheses for each K value (Figures 2, 3 and 4).

The Multi-cluster group included the following seven populations: *Thueringer Barthuehner*(#30), *Kastilianer*(#31), *Malay*(#32), *Gallus gallus gallus*(#33), *Malawi*(#34),

Godollo Nhx(#35) and *Orlov*(#36). It is rather non-trivial to point out historical information that could explain why these populations had membership in several clusters. Such an admixture can result either from being the descendant or the ancestor of several sources. The subspecies *Gallus gallus gallus*(#33) of the red jungle fowl is included among the admixed populations. Interestingly, while it is assigned to several clusters, it has almost no affinity with European and White Egg Layers' clusters.

A detailed structuring description among populations and clusters are presented as a supplementary section to this article.

Sub-clustering

The six clusters of the 2000 birds from 65 populations were each further sub-clustered by *STRUCTURE*, similarly to the approach that was applied for human data by Rosenberg et al. (2002) and Jakobsson *et al.* (2008). Following this strategy, most of the populations reached their final own distinct clusters that contained a single population.

European sub-cluster—Analysis of the European cluster was performed in two subsequent steps of sub-clustering; the first sub-clustering is marked as the subscript "1" and the second sub-clustering is marked as subscript "2". Except for *Westfaelische Totleger*(#17), the first sub-clustering gave consistent results for K equals $2_{(1)}$ and $3_{(1)}$ (Figure 3). One cluster (red strips) at $K=3_{(1)}$ represents breeds from the middle, east and south of Europe, while the other two clusters (pink and green strips; $K=3_{(1)}$) represent breeds from the north and west of Europe (Figure 3). The source of the distinction between these breeds from the middle, east and south European sub-cluster is unclear. Previous knowledge suggests that *Friesenhuehner*(#22) were crossed with Spanish breeds in the Netherlands after the Spanish occupation in the 16th century, and it is considered to be one of the ancestors of the following breeds: *Brakel*(#11), *Westfaelische Totleger*(#17), *Ostfriesische Moewen*(#25) and *Hamburger Sprenkel*(#23) (Dürigen 1885). The *Italiener Schwarz*(#14) breed was developed by crossing the Spanish breed *Minorka* with *Italiener rebhuhnfarbig* (Schwarz 1996). This information may explain why these breeds clustered together at $K=3_{(1)}$.

Further analysis of the European sub-cluster (Figure 3, $K=4_{(2)}$, right cluster) shows separation of the *Rheinlaender*(#28)and *Deutsche Sperber*(#21) , and of the *Friesenhuehner*(#22) and *Bergische Kraeher*(#19) from the other breeds. *Rheinlaender*(#28) is a Middle-West-European breed that was genetically influenced by the North-French breed *Le Mans* (Montag 1983), and thus it is differentiated from the other European breeds. The *Deutsche Sperber*(#21) was crossed with *Rheinlaender*(#28) at the end of the 20th century (Kämmerling 2002). Clustering of the *Friesenhuehner*(#22) and *Bergische Kraeher*(#19) agrees with earlier reports that these populations were imported from Southeast-Europe to Middle-Europe, centuries ago (Wandelt and Wolters 1996,Six 2005a,b).

Asian and other stocks—In further analysis of the 'broilers cluster', the commercial lines were first separated from the non-commercial populations, and at K=6, the commercial broilers were split into dam and sire lines (Figure 4A). Broilers originated from *White Cornish* (based on *White Asil*) and *White Rocks*, a breed with Asiatic origin. *Asil*(#48), a game type with pearl comb, was separated early in the clustering process from the rest of the non-commercial broiler populations which possess a single comb. Although the *New Hampshire*(#50) population is based on *Rhode Island Red*(#41), which is a brown egg layer, it has common breeding history with Yellow Indian Game (based on Asil, Schwarz 1996) that may explain its clustering within this group.

The Asian sub-cluster, when further analysed, separated into two distinct clusters at K=2 (Figure 4B). When K was increased to 5, these groups were separated into three and two

sub-clusters, respectively. Although *Brahma*(#55) and *Cochin*(#54) are breeds collected from German fanciers, they cluster with the Asian breeds because of their Chinese origin. At K=5, however, they make up their own group which may correspond to their isolation from native Chinese breeds. In addition, three multi-cluster populations were revealed within the main Asian cluster. The clustering of the Asian breeds at K=5 is in agreement with the clustering Chen et al. (2008) obtained for Chinese breeds.

The three populations in the No Common History cluster (NCH; Figure 4C) are from distinct genetic and geographical origins; to our best knowledge they didn't share any recent common ancestral origin. Thus, being clustered together indicates that these three populations are relatively far from all other populations in this study.

The White Egg Layers cluster (Figure 4D) contains only populations that have common breeding history with *White Leghorns*, which is the ancestor of the *white egg layers*. The *Bedouin*(#4) and *Iceland landrace*(#7) breeds which had low membership coefficients (0.61 and 0.44, respectively) with white egg layer cluster when the complete dataset was analyzed at K=6, were removed from the sub-clustering.

Sub-clustering the brown egg layers cluster (Figure 4E), first, differentiated the commercial egg layer lines from the *Rhode Island Red*(#41). The commercial lines were all separated clearly from each other when K values increased up to K=5.

In general, *STRUCTURE* differentiated well between populations. For example, *STRUCTURE* separated with minimal admixture even between commercial and experimental lines which managed as close populations for many generations. This verifies that *STRUCTURE* can efficiently utilize the SSR genotypic information for assessing population stratification. In the absence of such knowledge, the clustering result suggests previously unknown information about the genetic background of breeds.

Methodological Aspects - Repeatability of the results obtained by

STRUCTURE—In many cases *STRUCTURE* provides non identical results in repeated runs of the same data set. This is particularly true when analyzing a large number of populations with a moderate or large degree of complexity (Rosenberg *et al.* 2001, 2002; Parker *et al.* 2004). The algorithm may get stuck in a local optimum, returning results that do not reflect the true situation (Corander *et al.* 2004). This is due to the stochastic nature of the algorithm implemented in *STRUCTURE*. Similarity coefficient (C) can be used to assess the repeatability of runs on the same data set (Rosenberg *et al*. 2002). Parker *et al*. (2004) analyzed 414 dogs representing 85 pure breed populations, the similarity coefficients between runs of the same data were 0.84, 0.61 and 0.26 for 3, 4 and 5 clusters, respectively. Low similarity coefficients indicate low similarity between repeated runs of the same data set. Rosenberg *et al*. (2002) faced the same problem when analyzed a large human data set. In a preliminary study, when we increased the number of iterations up to 2.5 million (following the suggestion of the software authors), non-identical solutions have been observed in the repeated runs. In the current study we overcame this difficulty by taking the most common out of 100 solutions. We applied a procedure which adopts the most repeatable solution of *STRUCTURE*, using the similarity coefficient (C) (Rosenberg *et al.* 2002). The similarity coefficient was calculated for all possible 4950 pairs of 100 solutions generated by *STRUCTURE* for the same dataset. As was reported by Rosenberg *et al.* (2002) and seen in Figure 4, similarity coefficient (C) decreased rapidly when similarities between solutions are only slightly decreased. Thus, solutions with a pair wise $C \geq 0.95$ were considered to be identical. These solutions were clustered into groups of identical solutions and the most frequent solution was chosen to be the most accurate. The consistency between

most frequent solutions through subsequent K values is an indication that this is a satisfactory approach (Figures 1, 2 and 3).

AMOVA - between populations

Analysis of molecular variance (AMOVA) showed that the fraction of variance between breeds was about 34% (Table 2). This variation among chicken breeds is the highest reported for breeds of farm animals (MacHugh *et al.* 1998,Parker *et al.* 2004), and is very large in comparison with variation between human populations (approximately 12%; Li *et al*. 2008). High genetic diversity between breeds in farm animals is usually assumed to be due to barriers between breeds (Parker *et al.* 2004). In the current analysis we partitioned the variation into two components in a hierarchal manner: variation between clusters indicated by *STRUCTURE* software (Figure 2) and variation between breeds within these clusters. The results (Table 2) indicate that the variation between clusters is much lower (~11%) than the variation between breeds within clusters (~23%).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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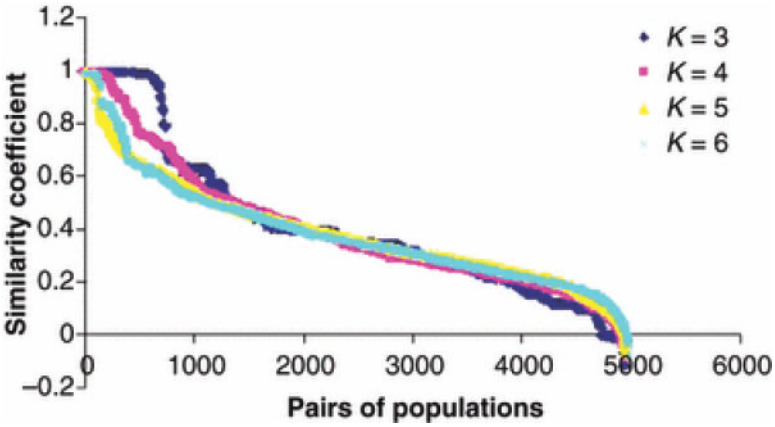

Figure 1.

Distribution of the similarity coefficients (C). The distribution was constructed on 4950 pairs of solutions which were obtained from 100 runs of *STRUCTURE* on the full data set at K=3 to 6. Pairs of solutions are ordered according to their similarity coefficients' (C) values in descending order.

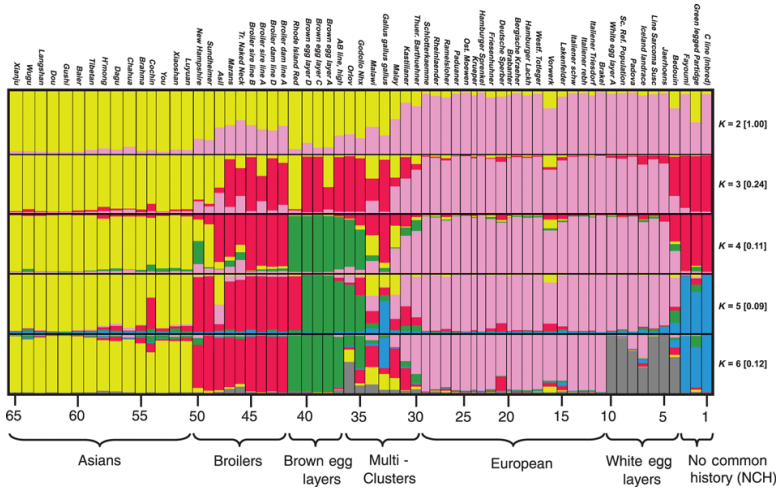

Figure 2.

Cluster patterns of 2000 individuals from 65 populations obtained by *STRUCTURE* based on 29 SSR loci, for varying number of clusters from K=2 up to K=6. For K=6 the clusters are: 1 - Brown Egg Layers, 2 – predominantly Broilers, 3 - native Chinese breeds or breeds with recent Asian origin, 4 - predominantly breeds of European derivation, 5 – a small cluster contains populations with No Common-History (NCH), and 6 - populations that had breeding history with *White Leghorn* including *white egg layers*. Another group of populations that shared their genome with several clusters was defined as “Multi-clusters”. The frequency of the presented solution among the 100 repeated solutions presented in quadrangular parentheses for each K value.

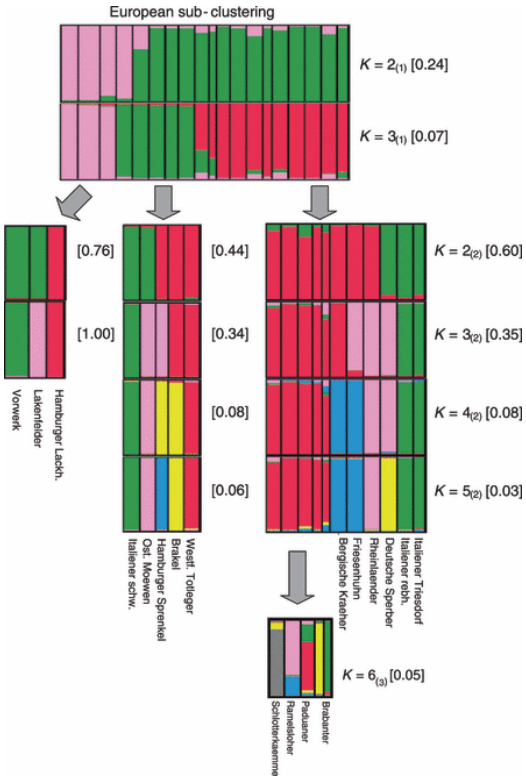

Figure 3.

First sub-clustering (marked with a subscript “1” in brackets) of the European populations resulted in three sub-clusters at K=3. Each of these three clusters was further separately divided into additional clusters in a second sub-clustering (marked with a subscript “2” in brackets). The frequency of the presented solution among the 100 repeated solutions presented in quadrangular parentheses for each K value.

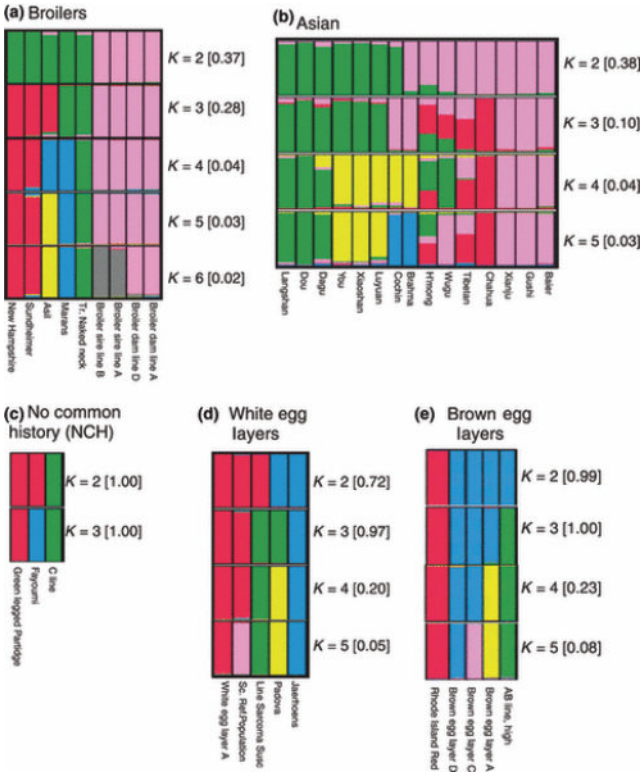

Figure 4.

Sub-clustering of the non-European main clusters: (A) Broilers, (B) Asians, (C) No Common-History (NCH), (D) White Egg Layers, and (E) Brown egg layers. The frequency of the presented solution among the 100 repeated solutions presented in quadrangular parentheses for each K value.

Table 1

List of the 65 populations used in this study

No.	Population	^{a}Cluster	Origin	Sampling country
1	*C line (Inbred)*	5	Inbred line	Czech Republic
2	*Green legged Partidge*	5	Mixed Type	Poland
3	*Fayoumi*	5	Mediterranean	France
4	*Bedouin*	6	Mediterranean	Israel
5	*Jaerhoens*	6	NW-European	Scandinavia
6	*Line Sarcoma Susc*	6	Mediterranean	Germany
7	*Iceland landrace*	6	NW-European	Scandinavia
8	*Padova*	6	Crested Fowl*	France
f9	*Sc. Ref. Population*	6	Mediterranean	Scandinavia
10	*White egg layer A*	6	Mediterranean	commercial
11	*Brakel*	4	NW-European	Germany
12	*Italiener Triesdorf*	4	Mediterranean	Germany
13	*Italiener rebh.*	4	Mediterranean	Germany
14	*Italiener schw.*	4	Mediterranean	Germany
15	*Lakenfelder*	4	NW-European	Germany
16	*Vorwerk*	4	NW-European	Germany
17	*Westf. Totleger*	4	NW-European	Germany
18	*Hamburger Lackh.*	4	NW-European	Germany
19	*Bergische Kraeher*	4	NW-European	Germany
20	*Brabanter*	4	Crested Fowl*	Netherlands
21	*Deutsche Sperber*	4	NW-European	Germany
22	*Friesenhuhner*	4	NW-European	Germany
23	*Hamburger Sprenkel*	4	NW-European	Germany
24	*Krueper*	4	NW-European	Germany
25	*Ost. Moewen*	4	NW-European	Germany
26	*Paduaner*	4	Crested Fowl*	Germany
27	*Ramelsloher*	4	NW-European	Germany
28	*Rheinlaender*	4	NW-European	Germany
29	*Schlotterkaemme*	4	NW-European	Germany
30	*Thuer. Barthuehner*	7	NW-European	Germany
31	*Kastilianer*	7	Mediterranean	Germany
32	*Malay*	7	Game	Germany
33	*Gallus gallus gallus*	7	Wild	Thailand
34	*Malawi*	7	African	Malawi
35	*Godollo Nhx*	7	Half Asian	Hungary
36	*Orlov*	7	Mixed type	Germany
37	*AB line, high*	1	Half Asian	Netherlands
38	*Brown egg layer A*	1	Half Asian	commercial
39	*Brown egg layer C*	1	Half Asian	commercial

No.	Population	[a]Cluster	Origin	Sampling country
40	*Brown egg layer D*	1	Half Asian	commercial
41	*Rhode Island Red*	1	Half Asian	Germany
42	*Broiler dam line A*	2	Half Asian	commercial
43	*Broiler dam line D*	2	Half Asian	commercial
44	*Broiler sire line A*	2	Half Asian	commercial
45	*Broiler sire line B*	2	Half Asian	commercial
46	*Tr. Naked Neck*	2	Mixed Type	Hungary
47	*Marans*	2	Half Asian	France
48	*Asil*	2	Game	Germany
49	*Sundheimer*	2	Half Asian	Germany
50	*New Hampshire*	2	Half Asian	Germany
51	*Luyuan*	3	Asian	China
52	*Xiaoshan*	3	Asian	China
53	*You*	3	Asian	China
54	*Cochin*	3	Asian	Germany
55	*Brahma*	3	Asian	Germany
56	*Chahua*	3	Asian	China
57	*Dagu*	3	Asian	China
58	*H'mong*	3	Asian	Vietnam
59	*Tibetan*	3	Asian	China
60	*Baier*	3	Asian	China
61	*Gushi*	3	Asian	China
62	*Dou*	3	Asian	China
63	*Langshan*	3	Asian	China
64	*Wugu*	3	Asian	China
65	*Xianju*	3	Asian	China

[a] clusters that were created using *Structure* software: **1** - Brown Egg Layers, **2** – predominantly Broilers, **3** – native Chinese breeds or breeds with recent Chinese origin, **4** - predominantly breeds of European derivation, **5** - a small cluster contains populations with No Common-History (NCH), **6** - populations that had breeding history with *White Leghorn* including *White Egg Layers*, and **7** – multi-cluster (breeds share several clusters)

* supposed origin is East Europe

Table 2

AMOVA based on 29 SSR loci of 65 chicken populations assigned into six clusters according to *Structure* analysis

Source of variation	Percentage of variation
Among clusters	11.16
Among populations within clusters	23.00
Within populations	65.84
Total	100