BREEDING AND GENETICS

Analysis of Genetic Relationships Between Various Populations of Domestic and Jungle Fowl Using Microsatellite Markers

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ABSTRACT The genomes of domestic and jungle fowl populations maintained in Ukraine and Germany were screened using microsatellites as molecular markers. Genetic variation and genetic distances between strains of different origins and performance potentials were determined. In total, 224 individuals of 20 populations were genotyped for 14 microsatellite markers covering 11 linkage groups. Of the 14 microsatellite loci, the number of alleles ranged between 2 and 21 per locus, the mean number of alleles being 11.2 per locus. By using Nei's standard distance and the Neighbor-Joining method, a phylogenetic tree was reconstructed; its topology reflected general patterns of relatedness and genetic differentiation among the chicken populations studied. Three major phylogenetic tree groupings were found. The red jungle fowl (*Gallus gallus*) formed a separate branch and demonstrated a specific allele distribution when compared with domestic fowl breeds analyzed. The second branch comprised commercial layer lines and chicken breeds that were subject to intense selection in the past or had common ancestral breeds with commercial strains. The third group encompassed the German native breed populations. The information about population and breed genetic relationships estimated by microsatellite analysis may be useful as an initial guide in defining objectives for designing future investigations of genetic variation and developing conservation strategies.

(*Key words*: chicken, jungle fowl, microsatellite, genetic diversity, phylogenetic relationship)

INTRODUCTION

During 8,000 yr of domestication, the chicken has been considerably changed and much differentiated by natural and artificial selections. The presumed ancestor of the domestic fowl, the red jungle fowl, lays 10 to 15 eggs per year in the wild, whereas commercial laying hens are capable of producing more than 300 eggs a year. Current breeding strategies for commercial poultry concentrate on specialized production lines derived by intense selection from a few breeds and very large populations with a great genetic uniformity of traits under selection (Notter, 1999). There are numerous other local and fancy breeds throughout the world that are characterized by medium or low performance and are often maintained in small populations. The genetic erosion of these local breeds may lead to the loss of valuable genetic variability in specific characteristics that are momentarily unimportant in commercial breeding strategies (Weigend et al., 1995). It can be assumed that local breeds contain the genes and alleles pertinent to their adaptation to particular environments and local breeding goals. Such local breeds are needed to maintain genetic resources permitting adaptation to unforeseen breeding requirements in the future and a source of research material (Romanov et al., 1996).

In the process of evaluating genetic diversity to develop conservation measures in chickens, it is of special interest to assess genetic variation between, on the one hand, domestic fowl stocks of different origins and performances including commercial lines and, on the other hand, jungle fowl populations, by utilizing modern molecular tools. Monolocus microsatellites have been shown to be suitable markers for this purpose (e.g., Vanhala et al., 1998; Weissmann et al., 1998; Zhou and Lamont, 1999) and may resolve phylogenetic relationships between closely related populations (Nei and Takezaki, 1996).

Abbreviation Key: ABG1, ABG2 = Australorp Black, two German populations; ABU = Australorp Black, Ukrainian population; BK1, BK2, BK3 = three Bergische Kräher populations; BS1, BS2, BS3 = three Bergische Schlotterkämme populations; L1, L2 = two commercial layer (Lohmann) lines; PCR = polymerase chain reaction; RW = Ramelsloher White; UB = Ukrainian Bearded; WT = Westfälische Totleger; YC = Yurlov Crower.

Among the various European native chicken breeds, there are some that are peculiar to Ukraine, Russia, and Germany that have not been included in genetic studies of more common commercial populations. One of the typical Ukrainian native breeds, Poltava Clay, originated in the mid 19th century from indigenous fowl presumably crossed to Buff Orpingtons. Since 1950, it has been intensively selected for egg production traits and resistance to neoplastic diseases and crossed to Rhode Island Reds (Romanov and Bondarenko, 1994). The endangered Ukrainian Bearded (or Ukrainian Ushanka) breed descended from local chickens in the mid 19th century but has not been subject to commercial selection for many decades. An old and endangered Russian breed, Yurlov Crower, is also thought to have been derived in the mid 19th century. It was once famous for long crowing ability (Moiseyeva, 1992; Romanov et al., 1996), but this trait has been lost in the present population. In Germany, there is another long crowing breed, Bergische Kräher. This breed was probably the result of crosses among breeds imported from Turkey (Vits, 1989, 1994), local German chickens, and birds brought by Spanish monks to Germany in the late 18th century (Wandelt and Wolters, 1996). The same German and Spanish roots probably gave rise to another breed, Bergische Schlotterkämme, resembling Mediterranean-type chickens in body shape. The Ramelsloher breed, known since 1874, descended from local Vierländer fowls crossed later to other breeds, including Cochin and Andalusian (Reber, 1994). The Westfälische Totleger have been distributed as local fowl in Westfalen since the early 19th century and have been recognized as a native breed since 1904 (Wandelt and Wolters, 1996). At present, the Bergische Kräher and Schlotterkämme, Ramelsloher, and Westfälische Totleger have endangered or critical risk status (http://www.fao.ovg/dad-is/). An exotic breed, Australorp Black, is distributed in Ukraine and Germany. This breed was derived from Black Orpingtons imported to Australia from England (May, 1982; van Wulfften Palthe, 1992).

The objective of the present study was to characterize and compare various populations of jungle and domestic fowls maintained in Ukraine and Germany. To achieve this target, 14 microsatellite loci were individually typed in 20 chicken populations. Based on this information, a dendrogram of breed differentiation was plotted, and phylogenetic relationships reflecting genetic divergence of captive jungle fowl, native breeds, and commercial lines were estimated.

MATERIALS AND METHODS

Experimental Populations

In total, 224 birds of 20 chicken populations kept in Ukraine and Germany were examined. The populations included in this survey were as follows:

Ukrainian (five populations): Ukrainian Bearded (UB), two selected Poltava Clay strains (P6, P14), Yurlov Crower (YC), and Australorp Black (ABU).

German (15 populations): three red jungle fowl populations [*Gallus gallus* (GG1, GG2, GG3)], three Bergische Kräher populations (BK1, BK2, BK3), three Bergische Schlotterkämme populations (BS1, BS2, BS3), Ramelsloher White (RW), Westfälische Totleger (WT), two Australorp Black populations (ABG1, ABG2), and two commercial layer lines (L1, L2).

The information on population origin, specific features, and number of individuals examined per population is presented in Table 1. All the Ukrainian populations used were kept at the Poultry Research Institute Collection Farm, Borky. The samples of German populations were obtained from fancy breeders and a commercial breeding company.[3]

DNA Isolation

One milliliter of venous blood was collected from the ulnar vein of each individual into 1.5-mL tubes with heparin or EDTA as anticoagulant. Blood samples were stored at −70 C. DNA was extracted from the whole blood by means of a QIAamp kit.[4]

Microsatellite Loci

The 14 microsatellite markers were selected from available databases on the World Wide Web for the Roslin Institute Chicken Genome Mapping Home Page (ChickGBASE, http://www.ri.bbsrc.ac.uk/chickmap/; Archibald et al., 1996) and the U.S. Poultry Genome Project Website (http://poultry.mph.msu.edu/) for the present study on the basis of linkage group representation (Table 2). Five of the microsatellite loci chosen, viz., MCW0004, MCW0001, MCW0005, MCW0014, ADL0158, have been recommended by the FAO/MoDAD Advisory Group (http://www.fao.org/dad-is); one locus, ADL0158, has also been included in the Population Tester Kit (U.S. Poultry Genome Project Website, http://poultry.mph.msu.edu/resources/poptest1.htm). One microsatellite marker, BNC1 (MCW0098), is a portion of a chicken DNA fragment that has 83% (93 nucleotides) identity to the human brain neuron cytoplasmic protein 1 gene (Wageningen Agricultural University Chicken Site, http://www. zod.wau.nl/vf/research/chicken/frame_chicken.html). Originally, seven more microsatellite markers were included in this study, LAMP1, LGALS4, OVY, PLN, ADL0136, HSPA3, and SRC; four of them are mononucleotide repeats. However, we did not succeed in obtaining reliable and scorable polymerase chain reaction (PCR) products for these markers due to 1) PCR amplification problems with some primers, 2) interference with true allele identification by additional stutter bands, or 3) difficulty in distinguishing alleles with 1 bp difference uniformly across populations. The last two problems have also been observed for chicken

TABLE 1. Description of the chicken populations used in this study

Population[1]	Origin	Specific features	No. of birds studied
UB	Ukraine	Medium-sized dual-purpose breed	10
P6	Ukraine	Synthetic dual-purpose line developed by crossing several Poltava Clay lines, purebred and crossbred (crossed to Rhode Island Reds), selected for egg production traits	10
P14	Ukraine	Selected for resistance to oncornaviruses	10
YC	Russia	Medium sized dual-purpose breed selected for long crowing, the trait being lost in the present population	10
ABU	Australia	Medium-sized dual-purpose breed	10
GG1	Southeast Asia	Wild fowl stock maintained in captivity	9
GG2	Southeast Asia	Wild fowl stock maintained in captivity	12
GG3	Southeast Asia	Wild fowl stock maintained in captivity	6
BK1	Germany	Medium-sized dual-purpose breed selected for long crowing	12
BK2	Germany	Medium-sized dual-purpose breed selected for long crowing	7
BK3	Germany	Medium-sized dual-purpose breed selected for long crowing	6
BS1	Germany	Mediterranean type breed	6
BS2	Germany	Mediterranean type breed	8
BS3	Germany	Mediterranean type breed	8
RW	Germany	Medium-sized dual-purpose breed	22
WT	Germany	Medium-sized dual-purpose breed	10
ABG1	Australia	Medium-sized dual-purpose breed	14
ABG2	Australia	Medium-sized dual-purpose breed	14
L1	Germany	Commercial layer line	17
L2	Germany	Commercial layer line	23

[1]UB = Ukrainian Bearded; P6, P14 = Poltava Clay strains P6 and P14; ABU = Australorp Black, Ukrainian population; YC = Yurlov Crower; GG1, GG2, GG3 = three red jungle fowl populations (*Gallus gallus*); BK1, BK2, BK3 = three Bergische Kräher populations; BS1, BS2, BS3 = three Bergische Schlotterkämme populations; RW = Ramelsloher White; WT = Westfälische Totleger; ABG1, ABG2 = Australorp Black, two German populations; L1, L2 = two commercial layer (Lohmann) lines.

mononucleotide repeat markers by Crooijmans et al. (1996a) and Vanhala et al. (1998).

PCR Procedure

The PCR products were obtained in 25 µL by using Ready-To-Go® PCR Beads[5] and a thermal cycler.[6] Between one and four pairs of microsatellite primers were run in one tube to perform single or multiplex reactions. Each PCR tube contained 50 ng of genomic DNA, 5 pmol of each forward primer labeled with IRD700 or IRD800,[7] 5 pmol of each unlabeled reverse primer, and 1 mM tetramethylammoniumchloride. The amplification protocol was an initial denaturation at 95 C (1 min), 35 cycles of denaturation at 95 C (30 s), primer annealing at temperatures varying between 55 C and 60 C (30 s), and extension at 72 C (1 min), followed by final extension at 72 C (10 min).

Specific DNA fragments produced by PCR amplification with microsatellite primers were visualized as bands by 8% PAGE, which was performed using a LI-COR automated DNA analyzer.[8] For calibration, an external molecular size ladder[7] was used. In addition, commercial internal size standards[7] or those amplified by the authors were included in each lane. Electrophoregram processing and allele size scoring were performed with the software package Diversity One.[9]

Statistical Analysis

Based on microsatellite allele frequencies, the phylogenetic relationships between populations were estimated using the computer software package PHYLIP (Felsenstein, 1994). Based on Nei's (1972) standard genetic distance and neutral mutation model, phylogenetic trees were reconstructed using the Neighbor-Joining method followed by the bootstrapping option with 1,000 resamplings.

RESULTS

Microsatellite Allele Distribution

For the 14 microsatellite loci examined, the total number of alleles was 157 across all populations, and an average of 11.2 ± 2.3 alleles per locus was calculated (Table 3). The number of alleles per locus ranged from two (BNC1) to 21 (MCW0005). The maximum size difference between the alleles observed within the loci ranged from 2 bp (in locus BNC1) to 108 bp (in locus MCW0119), with an average 32.9 bp per locus. Five markers (MCW0004, MCW0005, MCW0014, ADL0158, MCW0154) displayed size differences of 1 bp between some alleles. In particular, ADL0158 showed a series of seven alleles differing in size by only 1 bp (189 to 195 bp).

TABLE 2. Chicken microsatellite markers selected for the present study

Marker name <alias>	Chromosome or linkage group	Repeat sequence	Size range (bp)	Number of alleles described (n)
MCW0020	1	(TG)13[1]	(180–190)[1]	4[1]
MCW0004	3	(TG)28[1]	(159–199)[2]	7[3]
MCW0001	3	(TG)9[1]	(156–168)[2]	5[3]
MCW0116	3	(TG)11[1]	(283–289)[1]	2[2]
MCW0005	4	(TG)14[1]	(189–259)[2]	12[3]
BNC1 <MCW0098>	4	(TG)5TT(TG)7[1]	(260–262)[2]	3[1]
MCW0029	5	(TG)29[1]	(149–194)[2]	6[1]
MCW0014	6	(TG)18[1]	(164–188)[1]–190[4]	5[3]
MCW0165	E27C36W25W26	(CA)8[1]	(118–120)[2]	3[1]
ADL0158	E29C09W09	(CA)12[5]	(189–217)[5]	6[5]
MCW0123	E35C18W14	(AC)10[1]	(84–94)[2]	4[2]
MCW0119	E47W24	(CA)3N2(CA)8N2 (CA)8N4(CA)8[1]	(116–180)[1]	5[2]
MCW0104	E48C28W13W27	(TG)16[1]	(191–230)[1]	8[1]
MCW0154	Z	(CA)11[1]	170[6]–(171–193)[2]	6[2]

[1]Wageningen Agricultural University Chicken Site (http://www.zod.wau.nl/vf/research/chicken/frame_chicken.html).

[2]Crooijmans et al. (1996b).

[3]Crooijmans et al. (1996a).

[4]Vanhala et al. (1998).

[5]U.S. Poultry Genome Project Website (http://poultry.mph.msu.edu/resources/poptest1.htm).

[6]Weissmann et al. (1998).

In 11 of the 14 loci, a total of 27 alleles were found in the jungle fowl populations, which did not occur in any other population analyzed (Table 3). The size of these alleles, however, fell within the allele size range found across all populations studied, i.e. they seemed not to be clustered to one or the other end of the allele series. In addition, among the two major domestic fowl groupings established in this study (see Phylogenetic relationships section), 27 other nonshared alleles were determined between chicken populations belonging to one or the other group. In fact, we observed 15 and 12 alleles, respectively that were specific for certain selected lines and populations related to them or for German native breeds (Table 3).

Phylogenetic Relationships

Using Nei's (1972) genetic distance and the Neighbor-Joining method, a phylogenetic tree (Figure 1) was reconstructed for the chicken populations studied. The tree topology resulted in three major groupings, although the relationships between populations were not always supported by the bootstrap values. Three red jungle fowl pop-

TABLE 3. Microsatellite locus allele distribution

Marker name <alias>	Allele size range (bp)[1]	Most frequent allele (bp)	Observed no. of alleles
MCW0020	179–181–183–185	185	4
MCW0004	175–180–181[j]–182–185[G]–186 –188[j]–190[s]–196–201–205–21 2–214–216–218	186	15
MCW0001	158–160[j]–162–164–166–168–170[s]	166	7
MCW0116	275[j]–283–285	283	4
MCW0005	211–212–213–215[G]–218[s]–220–221–222–231–233–234–235–237–238[j]–239[j]–241–242[s]–244–247[s]–251[s]–252[j]	241	21
BNC1 <MCW0098>	256–258	256	2
MCW0029	140–146–148–150–152–154[j]–156[j]–162–164–168–170[j]–174[j]–183–185–187–189–191–193–195[G]–199[s]	187	20
MCW0014	173–175[s]–177[j]–178–179[j] –181[j]–182[j]–183–185–187–189[G]–204[j]	187	12
MCW0165	114–116–118	118	3
ADL0158	154[G]–174[s]–178–185–187–189–190–191–192–193–194–195[j]–203–209–214–222[j]	187	16
MCW0123	78[s]–80[G]–82–84[j]–86 –88–90–92–94	88	9
MCW0119	97[j]–113–115–117–119–131–133–143[G]–149–155[G]–157[G]–165 [G]–169[j]–177[s]–187[G]–205	115	16
MCW0104	189–192–194[j]–196–198[G]–200–202[s]–206–208–210–214[j]–220–222[s]–224[s]–226	189	15
MCW0154	169–170[s]–171[j]–175–179–180–181[j]–182–185–186–188[j]–190–192[j]	180	13

[1]Specific alleles for red jungle fowl are marked with the superscript j, for German native breeds with the superscript G, and for selected strains and populations related to them with the superscript s.
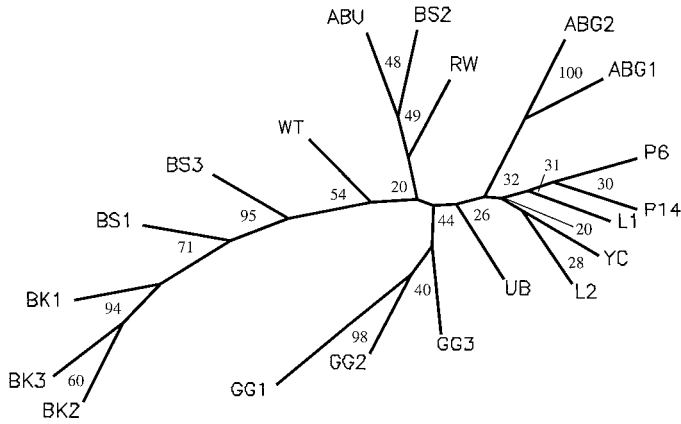
FIGURE 1. Dendrogram of phylogenetic relationships among 20 chicken populations. Consensus tree: numbers at the nodes are percentage bootstrap values from 1,000 replications with resampled loci. ABG1, ABG2 = Australorp Black, two German populations; ABU = Australorp Black, Ukrainian population; BK1, BK2, BK3 = three Bergische Kräher populations; BS1, BS2, BS3 = three Bergische Schlotterkämme populations; GG1, GG2, GG3 = three red jungle fowl populations (*Gallus gallus*); L1, L2 = two commercial layer lines; P6, P14 = Poltava Clay strains P6 and P14; RW = Ramelsloher White; UB = Ukrainian Bearded; WT = Westfälische Totleger; YC = Yurlov Crower.

ulations formed a separate branch. Two selected lines of the Poltava Clay chickens (P6, P14) and two German commercial layer lines (L1, L2) formed another branch. Close to them were the Russian native breed of YC, two German populations of ABG1, ABG2, and the UB. All native German breeds as well as ABU were grouped within the third major cluster, which consisted of two distinct branches, one comprising BK1, BK2, BK3, BS1, BS3, and WT, and the other including BS2, ABU and RW.

DISCUSSION

Microsatellite Allele Distribution

Compared to previously published data, our research revealed much greater microsatellite allele variation in chickens. In nearly all microsatellite loci analyzed, the number of alleles and their size range observed (Table 3) was greater than that reported by other authors (Table 2). This difference might be explained by the fact that, in our study, native chicken breeds from Germany and Ukraine and several jungle fowl populations were used that are unrelated to the chicken breeds included in these other investigations (e.g. Zhang et al., 1996; Takahashi et al., 1998; Vanhala et al., 1998; Zhou and Lamont, 1999). Relative to the chicken populations studied, we found that the jungle fowl populations constituted a specific microsatellite allele pool, which may demonstrate their phylogenetic unrelatedness to domestic fowl populations as expected from their breeding history. However, studies based on larger samples are needed to confirm this finding, because due to small number of individuals analyzed there is also a great likelihood that less common alleles were not found in other populations.

The expanded allele size distribution and number of alleles per locus will be useful information for application

in further microsatellite studies of chicken biodiversity. On the other hand, the authors are clearly aware that the observed differences in allele sizes among different laboratories might also be the result of analyzing microsatellite loci with equipment capable of different molecular size resolutions. Therefore, a common set of microsatellites to be typed and standard samples available as reference material would be desirable in future studies on chicken genetic diversity.

Seven of the microsatellite loci recommended by the FAO/MoDAD Advisory Group did not work in our hands. Following PCR amplification of the microsatellites, so-called stutter bands were observed in the electrophoresis gel. These are products amplified along with the major allele fragment; they are generally smaller and, in most cases, form a ladder with increments equal to the repeat unit length (LeDuc et al., 1995; Crooijmans et al., 1996a). In our densitometric analysis (data not shown), we observed that stutter peak height could be smaller than, equal to, or even larger than the major allele peak. On the other hand, their length could be 1 to 3 bp smaller or greater than that of the major fragment. This phenomenon complicated the scoring of microsatellite alleles, particularly where they differed by only 1 to 2 bp, and electrophoretic resolution was insufficient to separate the alleles from the stutter bands, as previously shown for mononucleotide repeat loci in chickens (Crooijmans et al., 1996a; Vanhala et al., 1998). These limitations must be taken into account when selecting markers for further microsatellite studies.

Because lane-to-lane variability was observed for the electrophoretic migration distances of the same alleles, we found it necessary to add two infrared dye-labeled markers as internal size standards to each lane. The sample allele sizes were then calculated, taking into account that allele migration relative to the two internal standards was constant. This approach enabled us to distinguish between alleles with 1-to-2-bp size differences and, in general, to eliminate the problem of inter- and intragel variation reported for polymorphic locus typing (e.g., Argüello et al., 1998).

The size distribution of microsatellite alleles that we observed (Table 3) did not completely correspond to that which one would expect from a stepwise mutation model. The observation of an irregular allele distribution in some of the loci examined supports the hypothesis that the structure of many microsatellites may not be simple, a conclusion reached by Freimer and Slatkin (1996), Barker et al. (1997), and Vanhala et al. (1998). The observed 1-bp differences between alleles in loci MCW0004, MCW0005, MCW0014, ADL0158, and MCW0154 might correspond to point mutations (deletions/insertions) in their flanking regions as it was reported for the CA repeats in the human HLA-DQ region (Lin et al., 1998).

Phylogenetic Analysis

Although the number of birds sampled for some populations was small, a lack of resolution in reconstructing the phylogenies of closely related populations was due to an

insufficient number of loci and the large number of populations studied rather than an insufficient number of samples per population as described by Shriver et al. (1995) and Chu et al. (1998). Nevertheless, to judge from the tree topology obtained (Figure 1), the resolution of our phylogenetic analysis was sufficient to reflect general patterns of the relatedness and genetic differentiation between the populations. Thus, three red jungle fowl populations formed a separate, ancestral cluster and demonstrated a specific allele distribution as compared to analyzed populations of domestic chickens. The selected Poltava Clay chickens and two German commercial layer lines, which may share some common Rhode Island Red genetic background, made up another major branch. The two Lohmann lines, L1 and L2, were genetically distinct as one would expect, because each line was selected differently in isolated populations without interbreeding for many generations.

The ABG1, ABG2 were grouped together and were quite close to selected lines within the second major cluster. For decades, the Australorp Blacks were widely exploited as dual-purpose commercial chickens. In 1950, this breed was imported to Germany and crossed to the Rhode Island Red, German Langshan, Barneveld, and New Hampshire breeds (Wandelt and Wolters, 1996). Therefore, the German Australorp Blacks, unlike the ABU, may have some of the same ancestral genes as those commercial breeds of the Rhode Island Red and New Hampshire, which have been used for creating such selected layer stocks as the Poltava Clay and Lohmann lines.

Two native breeds of Ukraine (UB) and Russia (YC) also map to the "commercial" branch. The YC chickens, probably derived from a Turkish long crowing breed, Denizli, or from mating some Chinese meat-type breeds, game breeds, and local Russian chickens, were famous not only for their long crowing ability but also for good commercial performance (Moiseyeva, 1992; Romanov et al., 1996). The UB chickens used to be widely spread in Ukraine and southern Russia (Moiseyeva, 1992; Romanov et al., 1996) and are observed to share some common alleles with the Poltava Clays and YC.

The third major cluster comprised the populations of German native breeds. Two Bergische Kräher flocks, BK2 and BK3, seemed to be of the same origin; the population BK2 has been kept unmixed for decades (W. Vits, 1999, Kolpingstraße 6, 35043 Marburg-Schröck, Germany, personal communication). The population BK1 is distinguished from two other Bergische Kräher flocks due to crossing with the Bergische Schlotterkämme. The common descent of the Bergische Kräher and Bergische Schlotterkämme has been confirmed by the combined grouping of BK1, BK2, BK3, BS1, and BS3 that displayed significant bootstrap values (Figure 1). The original Bergische Schlotterkämme became extinct in 1929 and were "restored" in the 1960s by crossing the Bergische Kräher and Silver Spangled Hamburg breeds to produce the population BS3, from which BS1 originated. Thus, the relatedness of current Bergische Kräher and Bergische Schlotterkämme flocks came into existence rather recently.

In contrast, the population BS2 has a different genetic background due to a cross with Castilian chickens (W. Vits, personal communication) that was verified by microsatellite diversity analysis. Although the grouping of BS2, ABU, and RW is quite heterogeneous and has insignificant bootstrap values, it is worth mentioning that the Australorp Black and RW have among their ancestors a common breed, Cochin (May, 1982; Reber, 1994), and the Ramelsloher White and population BS2 descended from two related Spanish breeds, Andalusian and Castilian. The affinity of the Westfälische Totleger to the Bergische Kräher and Bergische Schlotterkämme populations may be related to their narrow geographical localization in Germany and gene introgression due to crossbreeding, a common practice among fancy breeders (Reber, 1994; Vits, op. cit.). Noteworthy, the Australorp Black chickens from Germany and Ukraine as well as two so-called long crowing breeds, Bergische Kräher and YC, were genetically not similar, reflecting differences in their population histories.

The results of this survey demonstrate the usefulness of monolocus microsatellites as molecular markers to distinguish between different chicken populations and reconstruct quite plausible phylogenetic tree topology, even with a limited number of loci and samples analyzed and including situations where population histories are unclear. To our knowledge, this was the first time that the jungle fowl populations have been shown to have a specific microsatellite allele distribution distinct from domestic fowl populations. Also, there were some other nonshared alleles distinguishing two major domestic fowl branches, which may reflect a long independent history and development of these breeds in geographically distinct regions. The information resulting from this microsatellite analysis may be used as an initial guide to design further investigations of chicken genetic resources and for the development of conservation strategies.

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