Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science

Analysis of the genetic diversity of local Swedish chicken breeds using microsatellite markers

Abiye Shenkut Abebe

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ABSTRACT

The aim of the present study was to analyse the genetic diversity, genetic relationship and breed structure of ten local Swedish chicken breeds. A total of 127 chickens from ten breeds were genotyped using 24 autosomal microsatellite markers. Genotyping was performed by amplifying the genomic DNA using multiplex PCR kits and fragment analysis was carried out using genetic analyzer. A total of 124 alleles were detected in all populations, with a mean number of 5.17 alleles per locus. Within breeds, the observed and expected heterozygosities ranged from 0.219 to 0.417 and 0.231 to 0.559 respectively, and three breeds (Gotlandshöna, Hedemorahöna and Skånsk blommehöna) showed significant deviations from Hardy-Weinberg expectations. Similarly, 6 of the 24 loci were significantly deviated from Hardy-Weinberg. Inbreeding within breeds (F_{IS}) was generally high with the overall across loci and breed being 0.181. Due to small sample sizes in 50% of the breeds, the genetic relationship between breeds shown in the neighbor-joining tree was supported by low bootstrap values. The results of the breed structure analysis revealed that the Gotlandshöna, Hedemorahöna, Öländsk Dvärghöna and Svarthöna breeds formed their own distinct clusters, but breeds with small sample sizes (Skånsk blommehöna, Åsbohöna, Kindahöna, Ölandshöna, Gammelsvensk Dvärghöna and Orusthöna) clustered together. The results of the current study can be used as baseline genetic information that can be assimilated with genetic conservation programs, for instance, to control inbreeding within breeds and to implement further genetic studies in local Swedish chickens.

Key words: local chicken, genetic diversity, microsatellite locus, heterozygosity

1. INTRODUCTION

The domestic chickens (*Gallus gallus domesticus*) are known to be descended from the wild junglefowl population of the genus Gallus in Southeast Asia. The four known wild bird species of the genus Gallus, which are supposed to be the ancestors of the modern chickens, are the red junglefowl (*G.gallus*), the Sri Lankan junglefowl (*G. lafayetii*), the green junglefowl (*G. varius*) and the gray junglefowl (*G. sonnerati*) (Crawford, 1990; Moiseyeva et al., 2002). However, how many of these species and to what extent do they contributed to the ancestry of the domestic chicken is still unclear (Moiseyeva et al., 2002; Dessie et al., 2012). But, based on molecular data analysis (Akishinonomiya et al., (1994, 1996); Niu et al., 2002) and archaeological evidences (West and Zhou, 1988), it has been widely mentioned that the red junglefowl is the principal ancestor of the modern chickens. A recent study, however, revealed that the wild red junglefowl lacks the gene that controls yellow color in the skin, suggesting that the yellow skin observed in domestic chickens may be originated from other Gallus species which could be an evidence for multiple parental origins of the domestic chickens (Eriksson et al., 2008).

Following domestication events, chickens were spread across the globe mainly through trade and cultural exchange of peoples over several thousand years (Crawford, 1990; Storey et al., 2012). Such dispersion of chickens into different geographical areas and the subsequent adaptation to new environments, together with various selection pressures, had resulted in a considerable variation and breed differentiation of the domestic chickens (Romanov and Weigend, 2001; Food and Agriculture Organization of the United Nation (FAO), 2007). It is from these perspectives that many traditional chicken breeds, hereafter also called native or local chicken breeds, have existed worldwide. Local chickens are highly adaptive to their environment and can grow with minimal inputs; however, their productivity in general is very low (Romanov and Weigend, 2001; Kaya and Yildiz, 2008).

In response to the growing demand of high producing chickens and the availability of modern technologies, intensive selection and cross-breeding of some chicken breeds have been started since the middle of the 19th century (FAO, 2007). As a result of such a long period of breeding efforts, the commercial chicken lines which dominate the present poultry production sectors have been developed. The introduction of these lines, however, threatened the existence of native chicken breeds (Hillel et al., 2003; FAO, 2007; Chen et al., 2008). This may be because industrialization of chicken breeding favored the use of highly productive chicken breeds, and as a consequence led to the replacement of low performing native breeds (Granevitez et al., 2007). In addition, the majority of the genetic and phenotypic studies have focused on elite commercial chicken breeds kept in industrialized countries (FAO, 2011). Owing to poor commercial performances, native chicken breeds in many countries are not well characterized and far less attention has been given to genetic conservation of these resources compared to other livestock species such as cattle and sheep (Blackburn, 2006; FAO, 2007; Wilkinson et al., 2011).

In Sweden, there are eleven local chicken breeds, where ten of them are recorded as a breed in FAO local breed list (http://dad.fao.org/) and the remaining one breed is the one that was discovered recently in northern Sweden in a village called Bjurholm. Like the local breeds in different countries, most of the local Swedish chicken breeds became threatened by extinction when the commercially international breeds became more common, and typically only one or few populations with a small number of chickens remained when they were rescued by the Swedish association for local poultry (Svenska Lanthönsklubben) (personal communication, Anna Johansson). The association is still working on maintaining the local chickens in the form of live gene bank through its members and in collaboration with other partners (http://www.kackel.se).

Local Swedish chickens have been very little studied. Up to now, Bohuslän-Dals Svarthöna breed (mentioned throughout the paper with its common name Svarthöna) is the only local Swedish chicken breed that has been included in a published scientific study. Dorshorst et al. (2011) investigated the association of dermal hyperpigmentation phenotypes in chickens with structural variant observed in the genomic region that contains Endothelin 3 (END3) gene, and Svarthöna was among the breeds that showed phenotypic concordance with the structural variant. So far, there is no comprehensive information about the genetic diversity of local Swedish chicken breeds except with few morphological descriptions such as plumage color, body shape and sizes available at http://www.kackel.se. Thus, it is important to look at the genetic variability that might exist between breeds and among individuals within breeds in order not only to maintain the genetic resources but also could serve as potential sources for scientific studies in the future. For instance, hardiness in climatic variation, resistance to diseases, good mothering abilities, plumage and feather color variation, comb sizes and orientations are among the characteristics that could be investigated in local chickens.

Recent developments in molecular markers have provided multiple options to estimate the extent of genetic diversities within and across populations at the DNA level instead of differentiating chicken breeds based on their morphological appearance and feather colors. For example, estimating the level of heterozygosity and allele frequencies at different loci, estimating genetic distance between breeds, exploring the genetic structure of breeds and schematic representation of the existing genetic relationships can be made based on the information obtained from genetic markers (Vanhala et al., 1998; Hillel et al., 2007; Chen et al., 2008; Dávila et al., 2009). Currently, microsatellites are the most commonly used genetic markers to study genetic diversities in chickens as well as in other livestock species. This is because microsatellite markers are highly polymorphic as they contain multiple alleles per locus and show “co-dominant inheritance” in which each allele can express themselves at the same time (Hillel et al., 2003; Granevitez et al., 2007; Rajkumar et al., 2007). In addition, microsatellite markers are found to be abundant and evenly distributed throughout the genome with relatively high mutation rate (Li et al., 2002; Anmarkrud et al., 2008). So far, quite many studies have been conducted to assess chickens genetic diversity using microsatellite markers and the reported results are clear evidences of the usefulness of these panels for biodiversity studies (Hillel et al.,2003; Kaya and Yildiz, 2007; Wilkinson et al., 2011; Ramadan, et al., 2012;). For instance, Hillel et al. (2003) studied the biodiversity of multiple chicken breeds

including the red junglefowl, commercial lines and local chicken breeds of different countries; Kaya and Yildiz (2007) also analyzed the genetic diversity of two local Turkish chicken breeds.

The present study used data obtained from ten of the eleven local Swedish chicken breeds. Since these breeds are originated from different parts of Sweden and have been naturally selected for traits that fit their local environment, they might have possessed unique genetic characteristics which may contribute to the wide genetic diversity assumed to be found in natural populations, i.e. at least not intensively selected by human beings. Knowledge on the genetic diversity and breed structure firstly provides us with more insight about the differences and similarities between breeds and enables us to draw an overview of the current breeding practices, and secondly can be used as a basic input for possible future improvement of the breeds and to implement effective and sustainable breed conservation programs.

The objective of this MS thesis project was to study the genetic diversity, genetic relationship and breed structure of local Swedish chicken breeds using 24 microsatellite markers.

2. MATERIALS AND METHODS

2.1. Chicken samples

For the present study, a total of 127 local Swedish chickens obtained from ten breeds were available. Breed names and the number of chickens sampled from each breed were: Gotlandshöna (N = 33), Hedemorahöna (N = 36), Öländsk dvärghöna (N = 17), Skånsk blommehöna (N= 10), Ölandshöna (N= 4), Åsbohöna (N = 5), Kindahöna (N = 3), Gammalsvensk dvärghöna (N = 3), Svarthöna (N = 14) and Orusthöna (N = 2). Chickens were obtained from private owners involved in keeping of native chickens as a member of the Swedish local poultry association. All of the chickens grouped under the breed Ölandshöna, Åsbohöna, Kindahöna, Gammalsvensk dvärghöna and Orusthöna were sampled from one private owner (Lantrasparken), but kept as separate flocks. Chickens belonging to the Gotlandshöna and Hedemorahöna were obtained from three different sources, while chickens of the breed Öländsk dvärghöna, Skånsk blommehöna and Svarthöna were taken from two different chicken owners. Thus, the total chickens grouped into ten breeds were sampled from 17 different flocks. Sampling of chickens from a particular flock or source of any of the ten local chicken breeds was not random, rather family or flock members available at the time of sample collection were included. Nine commercial pure line chickens with known genotypes obtained from Germany were used as references for alleles calling.

2.2. Samples and DNA extraction

Blood samples collected from 127 local Swedish chickens were available. Genomic DNA was already extracted for most of the samples prior to the present study except 10 samples. For the 10 samples, DNA was extracted using QIAamp® DNA Blood Midi Kit (QIAGEN, Hilden, Germany). For all samples, measuring the amount of DNA was performed using thermo scientific NanoDrop^{TM} 8000 spectrophotometer. The purity of genomic DNA was assessed by observing the ratio of A260/A280 and A260/A230 which were calculated from the spectrums of ultraviolet-visible spectroscope absorbance measurements delivered by NanoDrop^{TM} 8000 spectrophotometer.

2.3. Microsatellite Genotyping

A total of 24 autosomal microsatellite markers were used to genotype the Local Swedish chickens and the reference samples included in this study. The markers were part of the 30 microsatellites recommended by the International Society of Animal Genetics (ISAG)-FAO to study the genetic resources of chickens at molecular level (FAO, 2011). Descriptions of the microsatellite loci including the primer sequences used in the present study are presented in Table S1 in Appendix 1.

Polymerase chain reaction (PCR) amplifications of the genomic regions encompassing the core motives of microsatellite loci were performed based on multiplex PCR techniques using the QIAGEN multiplex PCR kit (QIAGEN, Valencia, CA, USA). The 24 microsatellite markers were grouped into six multiplexes each with three to five pairs of primers per reaction plate. A final volume of 10 µl multiplex PCR master-mix contained: 10x PCR buffer with $MgCl_2$, dNTPs (25 mM), QIAGEN HotStar Tag DNA polymerase enzyme (5 U/µl), distilled water, fluorescently labeled forward and unlabeled reverse primers each with 10 µM

in concentration and genomic DNA (25 ng/µl). Amplification using Thermo-cycler was carried out with an initial incubation and enzyme activation of 95°C for 5 minutes, followed by 35 cycles of denaturation at 90°C for 30 seconds, primer annealing at 55°C for 45 seconds and extension of 72°C for 30 seconds, and lastly a final extension of 72°C for 15 minutes. All the multiplex PCR protocols and setups implemented in the present study are displayed in Appendix 2 through Table S2 to S5. After amplification, a reaction volume of 13 µl per individual sample was prepared by combining 1 µl of the amplicons and 12 µl from a mix of Formamide and GeneScan™ 600 LIZ® internal size standard. Following an initial heat treatment of 95°C for 3 minutes, capillary electrophoresis based fragment analysis was conducted using 3500xL genetic analyzer (Applied BioSystems) containing the pop_7™ (900) fragment analysis assay. The GeneMapper® 5 computer Software package (Applied BioSystems) was used to determine the fragment sizes and allele calling by comparing with known internal size standard.

2.4. Statistical analysis

2.4.1. Genetic diversity

Basic measures of genetic diversity, such as total number of alleles, allele frequencies, mean number of alleles, observed heterozygosity (HO) and unbiased gene diversity (HE) per locus and breeds were computed using FSTAT version 2.9.3 (Goudet, 2001) software package and an Excel Microsatellite Toolkit. Information polymorphic content (PIC), a measure of how microsatellite loci are informative in relation to expected heterozygosity (Guo and Elston, 1999), was calculated for each marker using Cervus version 3.0 software (Kalinowski, et al., 2007). Deviation from Hardy-Weinberg expectation per locus and breed was estimated and the significance of the test was assessed by performing 20,000 randomizations of alleles among individuals within breeds followed by sequential Bonferroni corrections of multiple testing employed in FSTAT software. Both Nei's (1987) fixation indices (G_{ST} and G_{IS}), and Weir and Cockerham (1984) fixation indices (F_{IT} (F), F_{ST} (θ) and F_{IS} (f)) per locus were estimated using FSTAT software. In the present study, sample size greatly varies between breeds, as a result Nei's and Weir and Cockerham fixation indices can result in different estimates. This is because Nei weights all samples equally, while Weir and Cockerham weight allele frequencies based on sample sizes. The significances of F_{IT}, F_{ST} and F_{IS} was determined by the 99% confidence intervals estimated from jackknifing over sample and loci, and bootstrapping over loci of permutation tests implemented in FSTAT software. The pairwise F_{ST} estimated between pair of breeds was used to assess the level of genetic differentiations among local Swedish chicken breeds.

2.4.2. Genetic contributions of breeds

The genetic contribution of breeds to the total genetic diversity was quantified following the method proposed by Carallero and Toro (2002). The method involves partitioning of the total genetic diversity into the within and between breed diversity. This was done by removing one or more breeds at a time from the population and then, quantifying the within and between breed diversities based on the molecular co-ancestry information using Molkin version 3.0 software (Gutiérrez et al., 2005). Positive contributions to diversity from any breed using Carallero and Toro (2002) method mean that the overall diversity increased because of the

remaining breeds, as a result, the assessed breed would be less preferred, for instance, for genetic conservations.

2.4.3. Genetic relationships

The genetic relationship of local Swedish chicken breeds was studied in two methods. First, Nei's (1972) genetic distance between pair of chicken breeds was estimated from allele frequencies and then bootstrapped 1000 times across all loci using PHYLIP version 3.9 software (Felsenstein, 2005). Subsequently, the neighbor joining method implemented in PHYLIP was used to construct the tree based on the genetic relationships generated in the distance matrices. Finally, the consensus phylogenetic tree was visualized using Dendroscope version 3.2.4 (http://dendroscope.org/). To examine the consistence of genetic relationships between breeds, for example, due to low bootstrap value and the presence of too few individuals in some breeds, a phylogenetic tree was reconstructed for five breeds by excluding breeds having fewer than ten individuals. Breeds excluded from the second phylogenetic tree were: Åsbohöna, Ölandshöna, Kindahöna, Gammelsvensk Dvärghöna and Orusthöna. A third phylogenetic tree was also constructed by splitting breeds into flocks. This is because some breeds such as Gotlandshöna and Hedemorahöna have more than one flock and each flock was sampled from different places. Thus, phylogenetic analysis based on flocks would enable to know uniformities within breeds.

In the second method, genetic structure of the studied chicken breeds was inferred from multi-locus genotype data using a Bayesian based approach employed in STRUCTURE version 2.3.4 software package (Hubisz et al., 2009). The analysis was carried out using an admixture model with independent allele frequencies between breeds (Pritchard et al., 2000; Hubisz et al., 2009). We ran the STRUCTURE analysis with an initial length of 20,000 burn-in periods followed by 100,000 MCMC (Marco Chain Monte Carlo) repeats for K (possible number of clusters) ranging from 2 to 10. For each value of K, 100 independent runs were performed. The STRUCTURE software generated a large file containing membership coefficients of all breeds and individuals which require reformatting of the matrices for further analysis. Thus, a web-based front-end program, STRUCTURE HARVESTER (Earl and vonHoldt, 2012), was used to organize the estimated cluster membership coefficients used in the downstream programs. Thereafter, pairwise comparisons of the 100 solutions generated for each value of K were carried out in a greedy algorithm implemented in CLUMPP version 1.1.2 software (Jakobsson and Rosenberg, 2007). Finally, clusters with the highest average pairwise similarity index (H) were converted into postscript file using DISTRUCT version1. 1 software (Rosenberg, 2004) and the different clusters were visualized with colorful graphics using Ghost view (http://pages.cs.wisc.edu/ghost).

The most likely number of clusters (Delta K) was calculated following the equation proposed by Evanno et al. (2005).

$$Delta\,\mathrm{K} = \mathrm{m}\left(\left|\mathrm{L}''(\mathrm{K})\right|\right)/\mathrm{s}[\mathrm{L}(\mathrm{K})]$$

where $m\left(\left|L''(K)\right|\right)$ was the mean absolute value of the second order rate of change of the estimated log likelihood of the data while $s[L(K)]$ was the standard deviation of the estimated log likelihood.

After identifying the most likely clustering number of K, according to Evanno et al. (2005), additional clustering analysis was performed for those breeds which showed mosaic clustering patterns for the most likely clustering number of K, i.e. K = 4, but uniformly clustered at K = 5 which was not considered as the most likely clustering according to Evanno et al. (2005). Breeds included in the second cluster analysis were: Ölandsk Dvärghöna, Åsbohöna, Skånsk blommehöna, Ölandshöna, Kindahöna, Gammelsvensk Dvärghöna, Svarthöna and Orusthöna. Subsequently, breed structure was analyzed for K values ranging from 2 to 6, keeping all parameters and procedures as similar as the first cluster analysis.

3. RESULTS

3.1. Genetic diversity within and between breeds

The number of alleles detected per loci and estimated value of various genetic diversity measures are presented in Table 1. Nearly all individuals were successfully genotyped for all markers except locus MCW0295 for two individuals, and loci MCW0069, MCW0014 and MCW0104, each for one individual, had missed genotypes. The four individuals with missing genotypes were found in three breeds.

Table 1. Number of genotyped chickens, number of alleles, observed heterozygosity (HO), between breeds (Hs) and overall (Ht) gene diversity, polymorphic information content (PIC) and Hardy-Weinberg (HE) per locus of ten local Swedish chicken breeds

Loci	Chickens	No. alleles	HO	Hs	Ht[1]	PIC	HW[2]
LEI0094	127	8	0.309	0.351	0.862	0.72	NS
ADL0268	127	6	0.284	0.485	0.718	0.62	NS
MCW0248	127	5	0.247	0.276	0.32	0.32	NS
MCW0216	127	4	0.280	0.370	0.592	0.49	NS
ADL0278	127	7	0.419	0.451	0.761	0.67	NS
MCW0295	125	8	0.233	0.451	0.846	0.73	*
MCW0081	127	7	0.453	0.456	0.730	0.6	NS
MCW0069	126	6	0.336	0.439	0.628	0.71	*
MCW0034	127	6	0.417	0.519	0.761	0.75	NS
MCW0222	127	4	0.284	0.233	0.277	0.22	NS
MCW0111	127	4	0.256	0.289	0.621	0.49	NS
MCW0037	127	5	0.790	0.583	0.664	0.58	NS
LEI0166	127	4	0.555	0.507	0.678	0.61	NS
ADL0112	127	4	0.248	0.317	0.600	0.58	NS
MCW0014	126	5	0.127	0.392	0.685	0.5	*
MCW0183	127	8	0.287	0.347	0.603	0.54	**
MCW0123	127	7	0.203	0.321	0.749	0.67	NS
MCW0165	127	4	0.073	0.296	0.714	0.58	*
MCW0020	127	4	0.491	0.511	0.781	0.68	NS
MCW0104	126	5	0.262	0.345	0.710	0.57	NS
MCW0078	127	4	0.477	0.348	0.498	0.35	NS
MCW0067	127	4	0.275	0.342	0.605	0.54	NS
MCW0330	127	3	0.243	0.420	0.655	0.58	*
MCW0098	127	2	0.297	0.327	0.467	0.37	NS
average	127	5.17	0.327	0.391	0.647	0.56	

[1] corrected for differences in sample size

[2] after Bonferroni correction: $P \leq 0.01$ = **, $P \leq 0.05$ = ** and NS = not significant

Markers polymorphism, estimated in terms of polymorphism information content (PIC), varied from 0.22 (MCW0222) to 0.75 (MCW0034) in the total population and the average was being 0.56. However, not all markers showed polymorphism in each of the ten breeds. Instead, 52 (of which 37 of them occurred in breeds having fewer than 10 individuals) of the 240 possible locus-breed combinations were found to be monomorphic (Appendix 3 Table S6 and S7). In the present study, a total of 124 alleles were identified in all breeds across the 24 microsatellite loci. The number of alleles per locus ranged from 2 (for MCW0098) to 8 (for LEI0094, MCW0295 and MCW0183) with an average number of 5.17 alleles (Table 1). The observed proportions of heterozygosities at different loci, in general, were low with the exception of MCW0037 and LEI0166 loci which contained an observed heterozygosity of 0.79 and 0.55 respectively. Heterozygosities observed at six loci were significantly deviated from Hardy-Weinberg expectations. Similarly, the overall observed heterozygosity and unbiased gene diversity (expected heterozygosity) across the 24 loci of all breeds were lower than the overall gene diversity of the population, i.e. average heterozygosity expected within the total population.

For each chicken breed, Table 2 shows the sample size, total number of alleles and various parameters used to measure genetic diversity within breeds. The number of alleles (shared and private alleles together) detected per breed ranged from 37 (for Orusthöna) to 76 (for Skånsk blommehöna). Similarly, the mean number of alleles per locus per breed varied from 1.54 to 3.17. The average heterozygosity observed in Ölandshöna, Svarthöna and Orusthöna was relatively low compared to the average heterozygosity observed in the other seven chicken breeds.

Table 2. Sample size, total number of alleles (TNA), mean number of alleles (MNA) per locus, observed (HO) and expected (HE) heterozygosities, test for Hardy-Weinberg (HW) and within breed inbreeding coefficient (F_{IS}) of ten local Swedish chicken breeds

breeds	Sample size	TNA	MNA/L	HO	HE	F_{IS}	HW[1]
Gotlandshöna	33	72	3	0.318	0.384	0.171	*
Hedemorahöna	36	66	2.75	0.306	0.401	0.238	*
Öländsk Dvärghöna	17	52	2.17	0.322	0.382	0.157	NS
Skånsk blommehöna	10	76	3.17	0.408	0.521	0.216	*
Ölandshöna	4	41	1.71	0.219	0.267	0.181	NS
Åsbohöna	5	58	2.42	0.417	0.48	0.132	NS
Kindahöna	3	58	2.42	0.403	0.559	0.28	NS
Gammelsvensk Dvärghöna	3	43	1.79	0.382	0.372	-0.027	NS
Svarthöna	14	45	1.88	0.225	0.231	0.028	NS
Orusthöna	2	37	1.54	0.271	0.302	0.103	NS

[1] after Bonferroni correction: $P \leq 0.05$ = * and NS = not significant

Only Gammelsvensk Dvärghöna breed showed no inbreeding, but in the rest of local Swedish chicken breeds the inbreeding coefficient varied from 0.028 (for Svarthöna) to 0.28 (for Kindahöna). Significant deviations from Hardy-Weinberg expectations were observed in three breeds (Gotlandshöna, Hedemorahöna and Skånsk blommehöna).

Table 3. Fixation indices estimated according to Nei's (1987) (G_{ST} and G_{IS}) and Weir and Cockerham (1984) (F_{IT} (F), F_{ST} (θ) and F_{IS} (f)) per locus across ten local Swedish chicken breeds

Loci	Nei's Fstatistics		Weir and Cockerham Fstatistics		
	G_{ST}[1]	G_{IS}	F_{IT} (F)	F_{ST} (θ)	F_{IS} (f)
LEI0094	0.593	0.120	0.536	0.456	0.088
ADL0268	0.324	0.415	0.663	0.502	0.285
MCW0248	0.136	0.108	0.267	0.074	0.208
MCW0216	0.375	0.243	0.616	0.432	0.305
ADL0278	0.408	0.069	0.532	0.507	0.063
MCW0295	0.467	0.483	0.553	0.336	0.312
MCW0081	0.375	0.007	0.392	0.264	0.195
MCW0069	0.300	0.234	0.604	0.410	0.335
MCW0034	0.317	0.196	0.435	0.384	0.088
MCW0222	0.160	-0.221	0.031	0.124	-0.101
MCW0111	0.535	0.113	0.569	0.486	0.167
MCW0037	0.122	-0.357	-0.145	0.182	-0.390
LEI0166	0.252	-0.095	0.568	0.550	0.053
ADL0112	0.472	0.215	0.652	0.597	0.130
MCW0014	0.427	0.676	0.880	0.326	0.834
MCW0183	0.425	0.173	0.664	0.548	0.284
MCW0123	0.572	0.369	0.608	0.502	0.213
MCW0165	0.585	0.752	0.862	0.691	0.601
MCW0020	0.346	0.039	0.480	0.388	0.149
MCW0104	0.514	0.240	0.842	0.813	0.158
MCW0078	0.301	-0.373	0.100	0.309	-0.302
MCW0067	0.434	0.197	0.746	0.617	0.304
MCW0330	0.359	0.422	0.680	0.435	0.478
MCW0098	0.300	0.093	0.287	0.259	0.052
overall	0.396	0.163	0.531	0.426	0.181

[1] corrected for differences in sample size between breeds

Fixation indices estimated per locus, according to Nei (1987) and Weir and Cockerham (1984), are summarized in Table 3. The G_{ST} and F_{ST} (both representing the fixation coefficient among breeds) values estimated for each locus were high and ranged from 0.122 (MCW0037) to 0.593 (LEI0094) and 0.074 (MCW0248) to 0.813 (MCW0104) respectively. The two parameters are supposed to provide similar estimates when breeds have equal sample size, but in the present study, sample size among breeds were not equal and estimates for the

majority of the loci were not similar. However, the overall fixation index across the 24 loci estimated using G_{ST} (0.396) and F_{ST} (0.426) were fairly similar. With the exception of locus MCW0037 (having an excess of heterozygosity, -0.145) and locus MCW0222 (with little amount of heterozygosity deficiency, i.e. 0.031), the global heterozygosity deficiency of individuals within the total population (F_{IT}) was very high for each locus and the average was being 0.531. The highest inbreeding coefficient of individuals, in both G_{IS} and F_{IS} estimator, was observed in locus MCW0014 and MCW0165. In both G_{IS} and F_{IS} estimator, inbreeding was not observed at three loci (MCW0222, MCW0037 and MCW0078); instead an excess of heterozygosity was shown in each of the three loci. The overall inbreeding coefficient observed across the 24 loci was 0.163 for G_{IS} and 0.181 for F_{IS}.

The 99% upper and lower confidence intervals of the average F_{ST} bootstrapped over loci were 0.494 and 0.357 respectively, indicating that the genetic differentiation between breeds was highly significant. Likewise, the average F_{IS} observed across the 24 loci was highly significant with 99% upper and lower confidence intervals of 0.313 and 0.043 respectively.

The fixation coefficient (F_{ST}) estimated between pair of breeds was generally high, but all the possible pairwise Fst comparisons of Gammelsvensk Dvärghöna and Orusthöna breeds did not show significant population differentiations (Appendix 3 table S8). The pairwise F_{ST} comparisons of Gotlandshöna, Hedemorahöna and Öländsk Dvärghöna breeds showed significant differentiations with all breeds except Orusthöna and Gammelsvensk Dvärghöna breeds.

Table 4. Loss or gain (%) of the total genetic diversity of local Swedish chickens when one of the breed was removed from the population based on the method developed by Caballero and Toro (2002)

Breeds	Genetic diversity	Within breed (%)	Between breed (%)	Loss (-) /gain (+) (%)
Gotlandshöna	0.594	-0.583	-2.824	-3.407
Hedemorahöna	0.596	-1.756	-1.401	-3.157
Öländsk Dvärghöna	0.602	-0.069	-2.163	-2.232
Skånsk blommehöna	0.612	-1.695	+1.116	-0.578
Ölandshöna	0.615	+0.736	-0.738	-0.002
Åsbohöna	0.613	-0.391	-0.033	-0.424
Kindahöna	0.612	-0.286	-0.263	-0.548
Gammelsvensk Dvärghöna	0.616	+0.267	-0.224	+0.042
Svarthöna	0.611	+2.937	-3.648	-0.712
Orusthöna	0.614	+0.386	-0.588	-0.202

3.2. Genetic contributions to the total diversity

The genetic contribution of breeds to the global genetic diversity was estimated following Caballero and Toro (2002), and the results are displayed in Table 4. The highest reduction of the global genetic diversity (-3.407%) was observed when the Gotlandshöna breed was removed. This is because of loss of the within (-0.583%) and between (-2.824%) breed diversity. When the Svarthöna breed was removed, the within breed diversity was increased (2.937%) but between breed diversity was decreased (-3.648%) and resulted in a global negative balance of -0.712%. In general, disregarding of either of the breeds except Gammelsvensk Dvärghöna had resulted in loss of the genetic diversity of the gene pool.

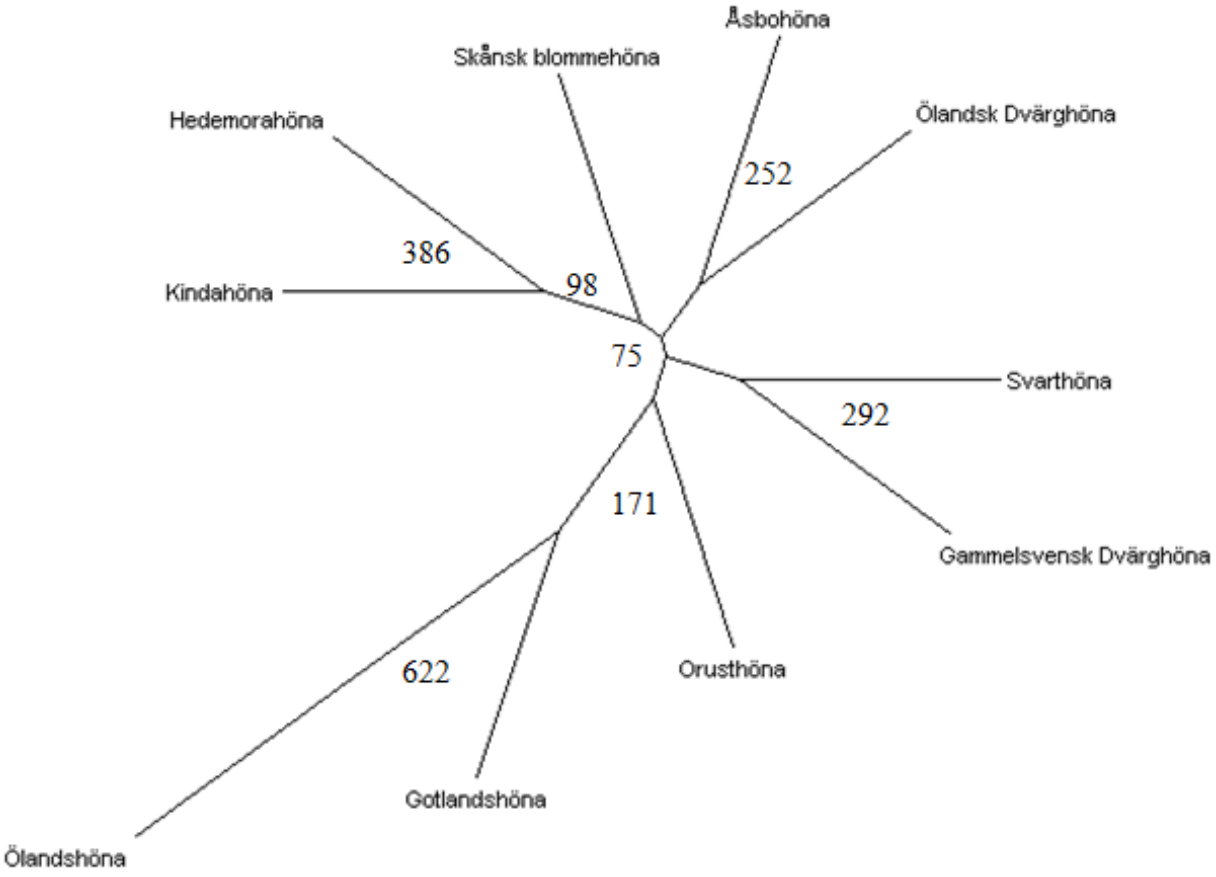

Figure 1. Unrooted neighbor-joining consensus tree constructed using Nei's genetic distance of ten local Swedish chicken breeds. The numbers on the branches show the frequency of occurrences of the associated branch from 1000 bootstrapping

3.3. Phylogenetic relationships

The neighbor-joining tree derived from Nei's genetic distance among ten local Swedish chicken breeds is given in Figure 1. The tree topology shows two broad clusters of the breeds: Kindahöna, Hedemorahöna, Skånsk blommehöna, Åsbohöna and Öländsk Dvärghöna in one cluster and the remaining five breeds into the second cluster. However, only the node that connects Ölandshöna and Gotlandshöna has shown relatively larger bootstrap value (62.2%) compared to nodes linking the rest of the breeds. Because of the absence of strong statistical evidences in each of the nodes linking the breeds, it might be difficult to infer the reliable phylogenetic relationships between breeds. When breeds with fewer than ten individuals

(Orusthöna, Kindahöna, Gammelsvensk Dvärghöna, Ölandshöna and Åsbohöna) were excluded from the phylogeny, the statistical power supporting the branching pattern of the tree was greatly improved (Figure 2). For example, in Figure 1, the genetic relationship between Hedemorahöna and Öländsk Dvärghöna was supported by very low bootstrap value (7.5%), but in Figure 2, their genetic relationship was supported by better statistical evidences of 83.3% bootstrap value. In both Figure 1 and 2, the genetic relationships between breeds were consistent. For instance, Gotlandshöna and Svarthöna, and Hedemorahöna and Skånsk blommehöna have a close genetic relationship in both Figure 1 and 2.

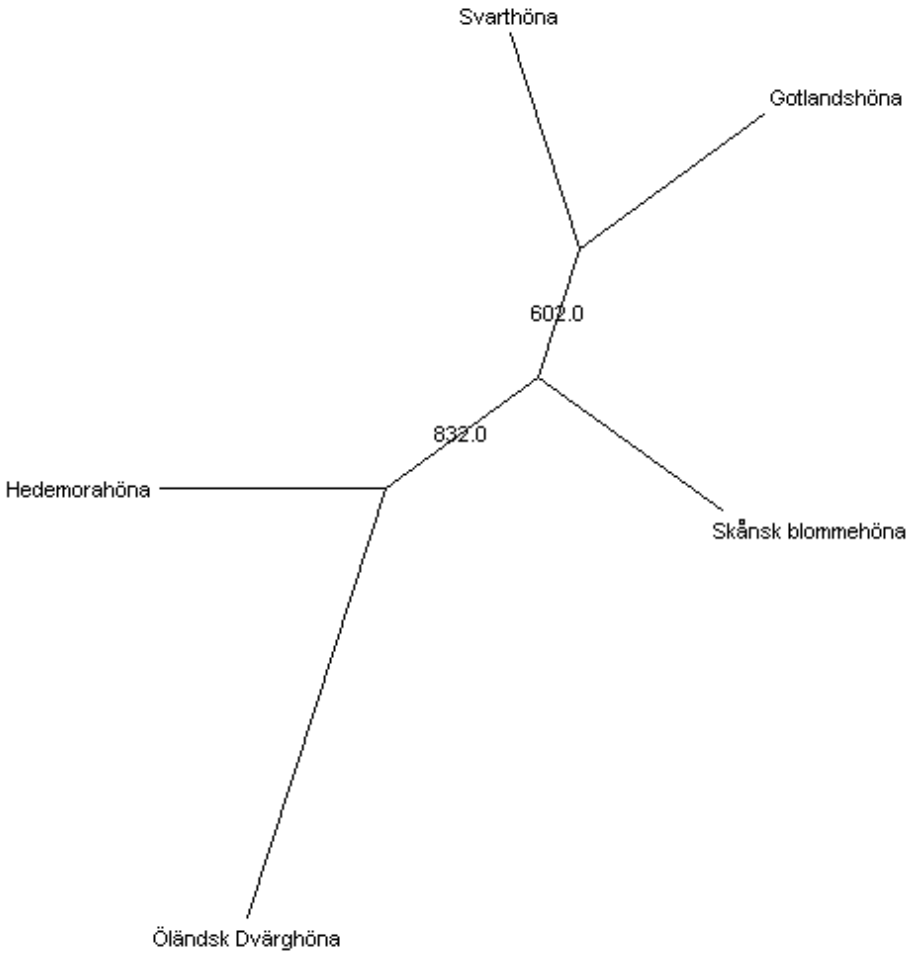

Figure 2. Unrooted neighbor-joining consensus tree constructed using Nei's genetic distance of five local Swedish chicken breeds with sample size greater than ten individuals. The numbers on the branches show the frequency of occurrences of the associated branch from 1000 bootstrapping

The genetic relationship of ten local Swedish chicken breeds was further studied by splitting breeds into flocks (Figure 3). According to Figure 3, one flock from Gotlandshöna and one flock from Skånsk blommehöna were not assigned into their respective breeds, though the statistical evidences were low, this may indicate the presence of population admixture and migrant individuals. Flocks of the Öländsk Dvärghöna, Hedemorahöna and Svarthöna were properly grouped into their breeds with sufficient evidences. For instance, the two flocks of Svarthöna breed appeared together in 99.9% of the time.

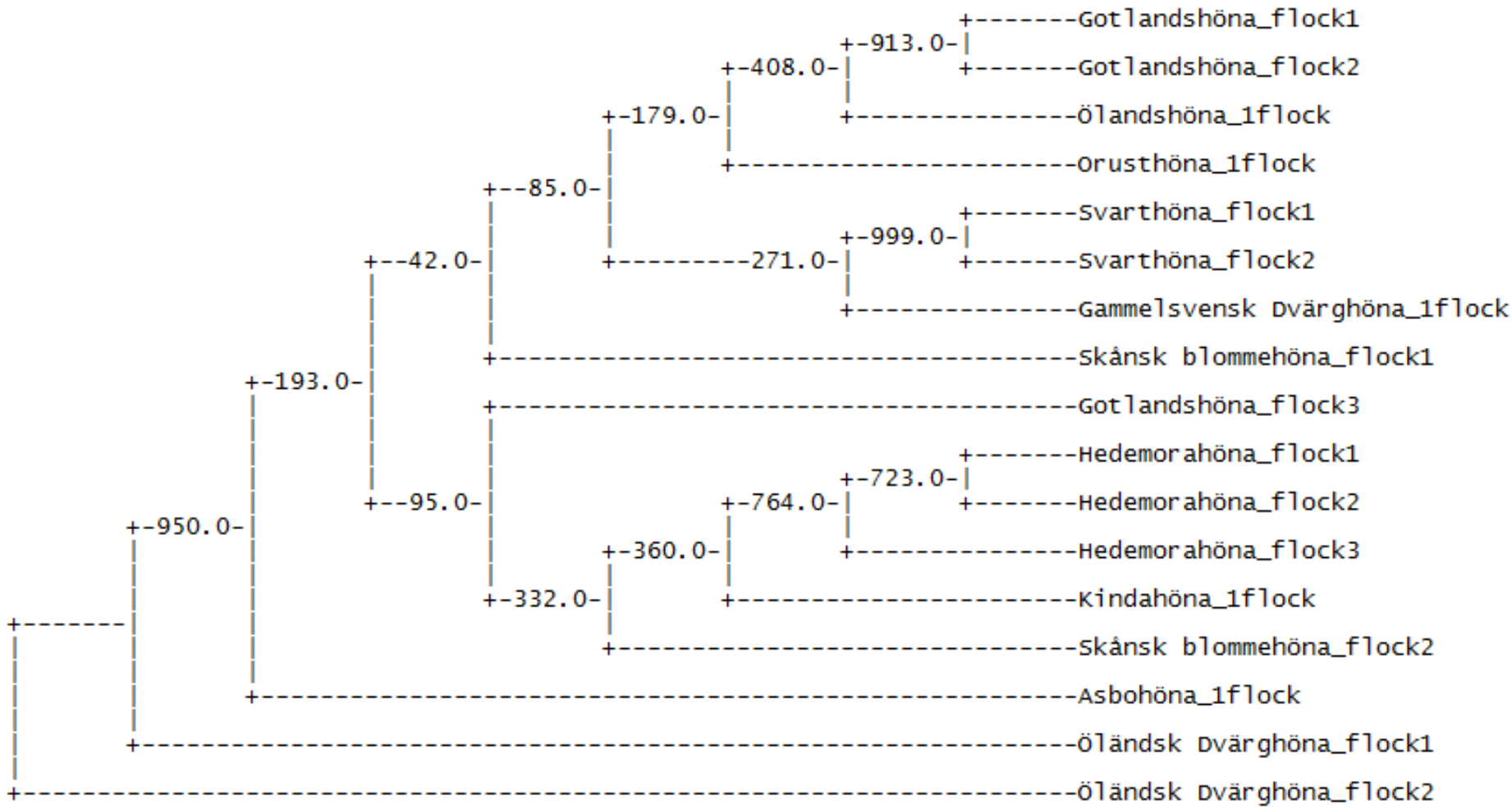

Figure 3. Neighbor-joining rectangular cladogram constructed using Nei's genetic distance between seventeen flocks of the ten local Swedish chicken breeds. The numbers on the branches show the frequency of occurrences of the associated branch from 1000 bootstrap

3.4. Breed structure and individual's assignment

The results of the STRUCTURE clustering are shown in Figure 4. When low value of K (i.e. K= 2) was assumed, individuals from Gotlandshöna, Svarthöna and Orusthöna were grouped into one cluster while individuals from the breed Hedemorahöna and Ölandshöna had covered the largest portions of the second cluster. However, individuals from the remaining five breeds shared both clustering patterns. At k = 3, Gotlandshöna and Hedemorahöna breeds showed distinct clusters leaving the other eight breeds as a mixed cluster.

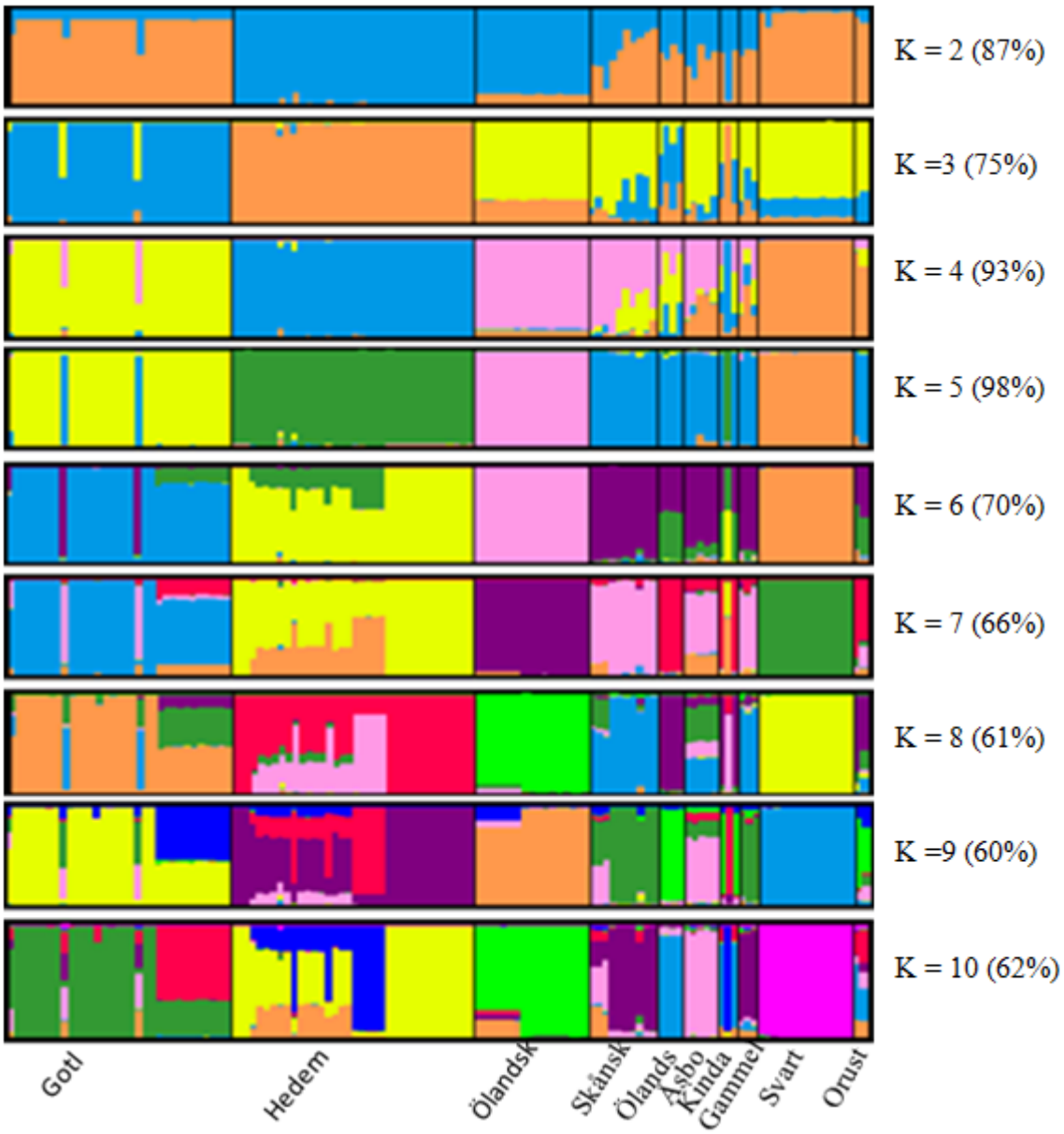

Figure 4. STRUCTURE Clustering of ten local Swedish chicken breeds: Gotl = Gotlandshöna; Hedem = Hedemorahöna; Ölandsk = Öländsk Dvärghöna; Skånsk = Skånsk blommehöna; Ölands = Ölandshöna; Åsbo = Åsbohöna; Kinda = Kindahöna; Gammel = Gammelsvensk Dvärghöna; Svart = Svarthöna. Numbers in the parenthesis indicate the average similarity index between individuals assigned into the same cluster

Subsequently, at K = 4 and 5, Gotlandshöna, Hedemorahöna, Öländsk Dvärghöna and Svarthöna breeds were placed into separate clusters, whereas the other six breeds showed up mosaic clustering patterns at K = 4, but were uniformly clustered together at K = 5. The average similarity index between individuals assigned into the respective clusters was significantly high at K = 4 and 5. Few individuals in the Gotlandshöna and Kindahöna breeds were consistently deviated from their breed's cluster at all values of K, indicating that they were admixed. At K = 6 and above, lack of uniformity between individuals assigned into the same cluster and reductions of the corresponding average similarity index between individuals were markedly observed.

The most likely number of clusters of the local Swedish chickens included in the present study was calculated based on the method developed by Evanno et al. (2005). The highest Delta K (4.407), calculated by dividing the absolute value of the second order rate of change of the log likelihood of the data (|Ln"(K)|) to the standard deviation of the log likelihood, was found at K = 4 (Table 5 and Figure 6), indicating that K = 4 was the most probable number of clusters of the ten local Swedish chicken breeds. However, at K = 5, the value of Delta K was not too far from K =4 unlike the others. In addition, individuals were assigned into clusters more uniformly with the highest similarity index (98%) at K = 5.

Table 5. Values of K, the number of repeats for each K, mean log likelihood and standard deviations of the data (LnP(K)), the mean difference between consecutive likelihood values of K (Ln'(K)), absolute values of the second order rate of changes of the likelihood |Ln''(K)| and the most likely number of clusters (Delta K) of ten local Swedish chicken breeds calculated according to Evanno et al. (2005)

K	Repeats	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
2	100	-6048.15	114.185	—	—	—
3	100	-5453.82	71.481	594.333	140.252	1.962
4	100	-4999.74	53.090	454.081	233.946	4.407
5	100	-4779.6	44.207	220.135	137.794	3.117
6	100	-4697.26	19.556	82.341	12.496	0.639
7	100	-4627.42	18.122	69.845	26.251	1.449
8	100	-4583.82	29.107	43.594	12.482	0.429
9	100	-4552.71	44.690	31.112	11.976	0.268
10	100	-4533.57	41.510	19.136	—	—

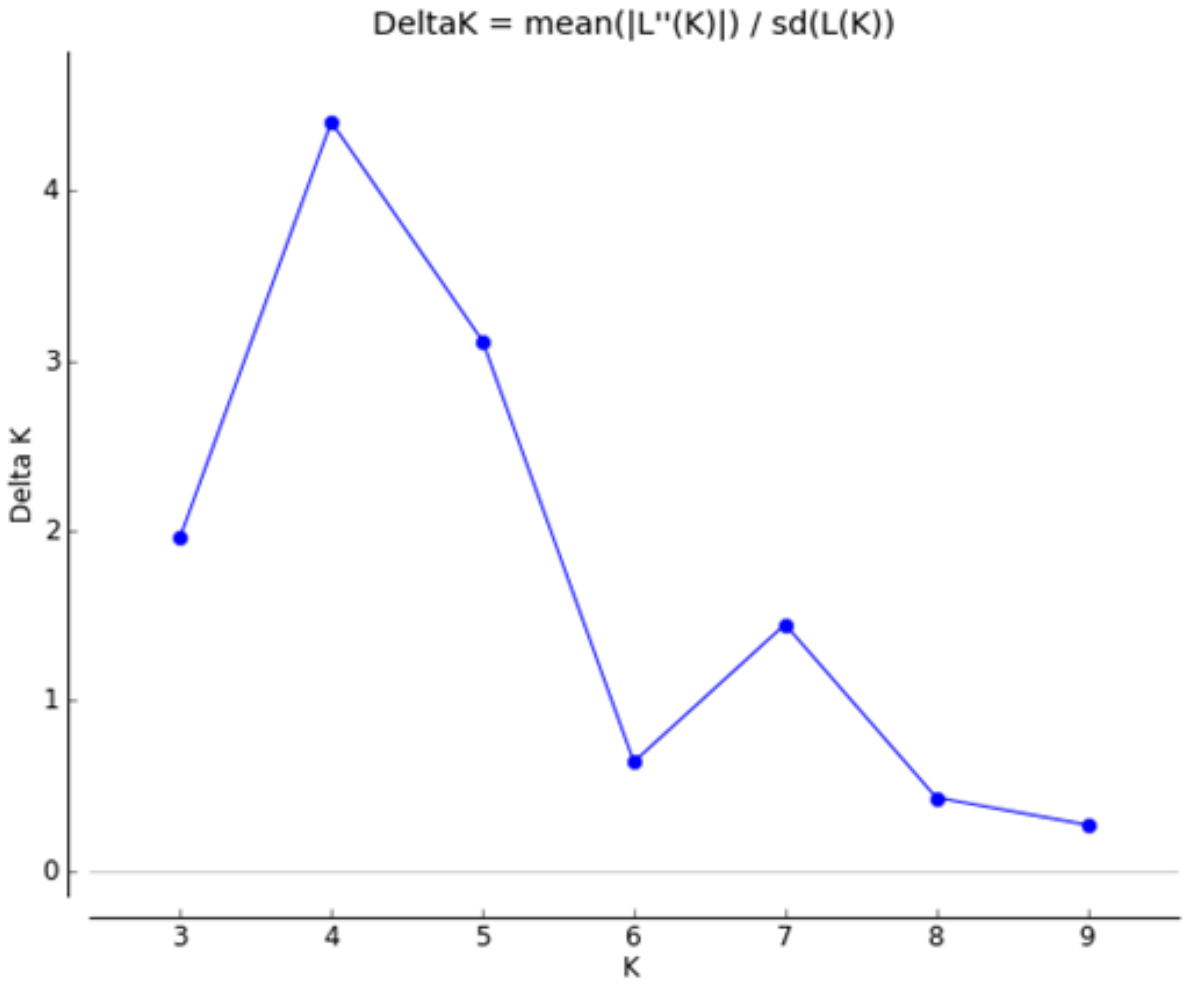

Figure 5. A scatter plot of the different values of K vs Delta K, and the highest peak shows the most likely number of clusters of local Swedish Chickens included in the present study

To sort out such ambiguity, STRUCTURE analysis encompassing eight breeds (excluding Gotlandshöna and Hedemorahöna) was again conducted with K running from 2 to 6. The results of the second STRUCTURE analysis (Figure 6 and Table 6) showed that at K = 3 individuals of the eight breeds were assigned into 3 clusters with an average similarity index of 99.9%. At K = 3, individuals in the Öländsk Dvärghöna and Svarthöna breeds were assigned into separate clusters, but individuals in the remaining six breeds (Skånsk blommehöna, Ölandshöna, Kindahöna, Åsbohöna, Gammelsvensk Dvärghöna and Svarthöna) were uniformly kept in one cluster. The highest Delta K (216.046) was found at K = 3, showing that the most possible number of clusters of the eight breeds was 3 (Table 6). The clustering patterns observed at K =3 in Figure 6 and k = 5 in Figure 5 were quite similar. In the second STRUCTURE analysis at K = 4 and above, lack of clustering uniformity of individuals and decreased similarity index between individuals were observed.

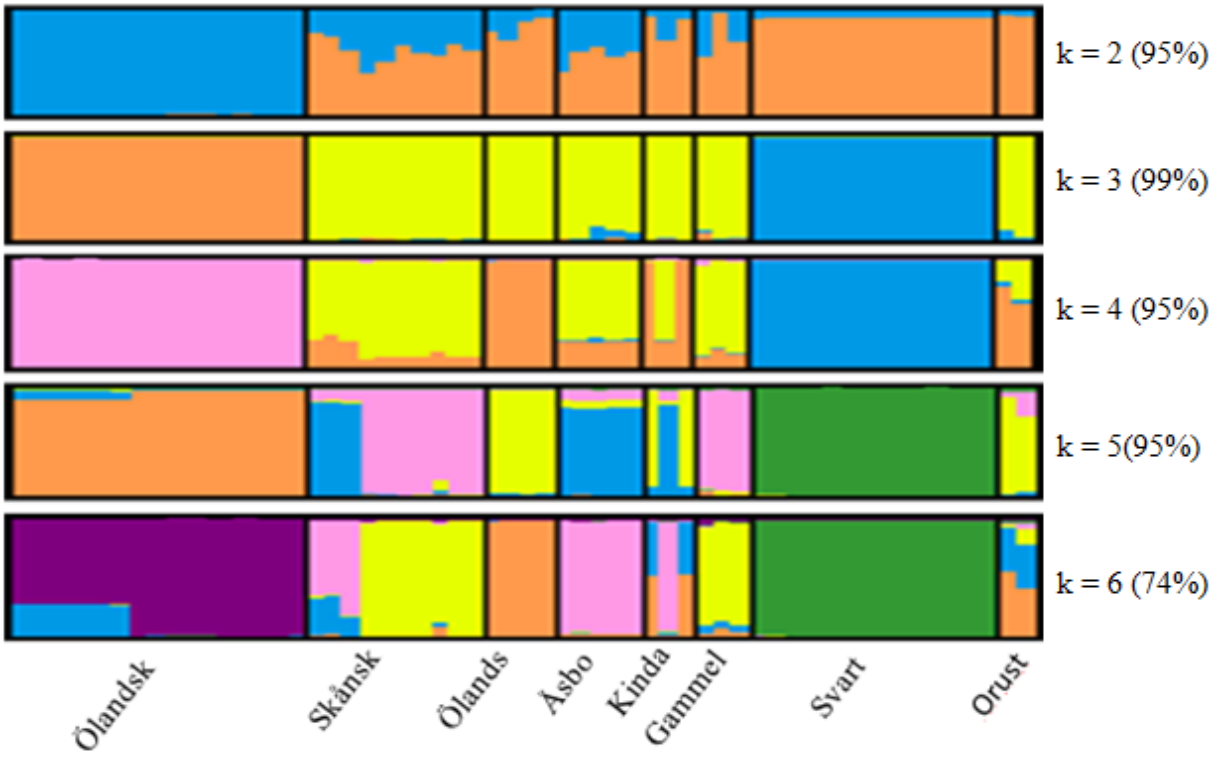

Figure 6. STRUCTURE clustering of eight chicken breeds: Ölandsk = Öländsk Dvärghöna; Skånsk = Skånsk blommehöna; Ölands = Ölandshöna; Åsbo = Åsbohöna; Kinda = Kindahöna; Gammel = Gammelsvensk Dvärghöna; Svart = Svarthöna; Orust = Orusthöna. Numbers in the parenthesis indicate the average similarity index between individuals assigned into the same cluster

Table 6. Inferring the most likely number of clusters (Delta K) in eight local Swedish chicken breeds. Parameters were estimated according to Evanno et al. (2005)

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
2	100	-2690.86	33.951	—	—	—
3	100	-2424.64	0.792	266.214	171.167	216.046
4	100	-2329.59	5.848	95.047	37.511	6.414
5	100	-2272.06	44.303	57.536	31.274	0.706
6	100	-2245.8	10.847	26.262	—	—

4. DISCUSSION

4.1. Genetic diversity within and between breeds

In the present study, we have investigated the genetic diversity and phylogenetic relationships existing within and between ten local Swedish chicken breeds by genotyping 127 chickens at 24 microsatellite markers. The average observed and expected heterozygosities which reflect the within and between breeds genetic diversity were quite low, indicating that loss of heterozygosity in local Swedish chicken breeds is relatively high. This was evidenced by the presence of high inbreeding, ranging from 0.103 to 0.28 in at least 80% of the breeds and low mean number of alleles per locus and breed (Table 2). This may be associated with small effective population size of breeds and breeding practices such as assortative mating implemented by owners keeping local chickens. Particularly keeping breeds with small isolated flocks and small population size over many generations would result in loss of heterozygosity due to the high chances of random genetic drift and inbreeding (Young and Clarke, 2000).

The presence of monomorphic markers (see Appendix 3 Table S6 and S7) could also account for the reduction of heterozygosity and inflate inbreeding estimates. However, all the microsatellite markers included in the present study are members of the 30 loci recommended by ISAG-FAO for genetic diversity studies in chickens, and their polymorphism characteristics have been proven in various genetic diversity studies of chickens (e.g. Hillel et al., 2003; Muchaddeyi et al., 2007; Ramadan et al., 2012). Sampling of genetically related individuals, particularly the inclusion of more number of chickens from the same flock, may contribute to the existence of monomorphic markers but can provide good opportunities to evaluate the past and current breeding practices especially in controlling inbreeding within the population. Similar to the present study, Zanetti et al. (2010) reported low heterozygosity estimates in six local Italian chicken breeds using 20 microsatellite markers and population founder effects are mentioned as the possible factors accounted to low genetic diversity. Other studies (e.g. Muchaddeyi et al., 2007 in local Zimbabwe chickens; Kaya and Yildia, 2008 in local Turkish chickens; Cuc et al., 2010 in local Vietnamese chickens) reported relatively larger heterozygosity estimates for different local chicken breeds using similar microsatellite loci. This could be due to genetic variations between breeds and differences in breed management at different countries. The average number of alleles detected per locus (5.17) in the present study was similar with local Italian chickens reported by Zanetti et al. (2010), but lower than estimates reported by Hillel et al. (2003) for 52 chicken breeds sampled from different countries and Chen et al. (2008) for 15 local Chinese chicken breeds. Unlike the present study, the latter two reports were based on large sample sizes.

Although the overall heterozygosity, measured per breed across the 24 loci and per locus across ten breeds, in general is low, differences in genetic diversity between breeds and loci existed. For instance, the average observed and expected heterozygosities at loci MCW0037 and LEI0166 were much larger than the corresponding estimates at MCW0165 and MCW0014 loci (Table 1). The latter two loci showed significant deviations from Hardy-Weinberg expectations and the within population inbreeding coefficients, G_{IS} (Nei, 1987) and F_{IS} (Weir and Cockerham, 1984), were considerably high (Table 3). Excess homozygosity or

heterozygosity deficit observed in some of the loci was not just simply due to fixation of one type of allele in all breeds; instead it was due to the presence of different homozygote alleles for different breeds at the same locus. For example, the total number of alleles observed in all genotyped individuals at MCW0014 and MCW0037 were equal, i.e. 5 in both loci, but heterozygosity estimates were not the same. Excess homozygosity could also occur due to null allele effects, a situation in which the genotyping assay failed to detect alleles due to mutations in the primer binding sites (Dakin and Avise, 2004). The presence of null alleles may cause locus specific heterozygosity deficits and mismatches in known parent-offspring relationships (Castro et al., 2004; Dakin and Avise, 2004; Wilkinson et al., 2011). In the present study, three chickens (two from Hedemorahöna and one from Gotlandhöna) had single parent information obtained from the owners at the time of sample collection. Based on the parentage information, MCW0014 locus showed parent-offspring mismatches in two chickens (one from Hedemorahöna and one from Gotlandhöna). In addition, high inbreeding and deviation from Hardy-Weinberg equilibrium was observed at MCW0014 locus. Taking this into consideration, null allele effect may exist at this particular locus. However, in the previous studies, the effects of null alleles in any of the 30 microsatellite loci recommended by ISAG-FAO are not reported (Muchaddeyi et al., 2007; Granevitze et al., 2007; Zanetti et al., 2010; Ramadan et al., 2012).

The level of genetic diversity also varies between breeds. Kindahöna and Åsbohöna breeds have shown more genetic diversity than Svarthöna breed (Table 2). This may be because, apart from breed differences in genetic diversity, 9 of the 24 loci were fixed in Svarthöna population which could affect estimates of heterozygosity and inbreeding. To minimize biases in heterozygosity estimates due to differences in sample size between breeds, Nei's (1987) unbiased estimator of heterozygosity was applied. However, variations in sample size may still have effects on parameter estimates. For example, most of the monomorphic markers were observed in breeds having fewer than ten individuals such as Orusthöna, Gammelsvensk Dvärghöna and Ölandshöna breeds. The Skånsk blommehöna breed has shown better genetic diversity than all breeds but still with high inbreeding which is significantly different from zero. Inbreeding coefficient estimated for Gammelsvensk Dvärghöna and Svarthöna breeds was very low, but it is unlikely, because both observed and expected heterozygosities were low and some microsatellite markers were monomorphic for these breeds. Three breeds (Gotlandshöna, Hedemorahöna and Skånsk blommehöna) showed significant deviations from Hardy-Weinberg expectations. Local Swedish chickens have not been selected for specific traits. Thus, deviations from Hardy-Weinberg may be due to small population size and nonrandom mating within the breed.

4.2. Genetic differentiation between breeds

Fixation indices, G_{ST} (Nei, 1987) and F_{ST} (Weir and Cockerham, 1984), estimated per locus were relatively high, indicating a high degree of genetic differentiation between breeds (Table 3). The 99% confidence intervals of the overall F_{ST} of all loci (0.357, 0.494) also confirm the presence of significant genetic differentiation between breeds. Despite differences in sample size between breeds, mean values of G_{ST} (0.396) and F_{ST} (0.426) estimated across the 24 loci were fairly similar but values at different loci for the two

parameters were not the same. This is because Nei weights all samples equally, whereas Weir and Cockerham weight allele frequencies based on sample sizes and as a result, the two parameters may give different estimates when sample size varies between breeds. The level of genetic differentiation estimated in the present study was approximately equivalent to mean F_{ST} value of 0.437 reported by Zanetti et al. (2010) using 20 microsatellite markers in Italian chicken breeds. Similarly, Tadano et al. (2007) also reported high F_{ST} value (0.429) for seven Japanese native chicken breeds using 40 microsatellite loci. However, Eltanany et al. (2010) reported very low genetic differentiation (mean F_{ST} = 0.07) between ten Egyptian native chicken breeds using 29 microsatellite loci. Likewise, Dávila et al. (2009) reported relatively low genetic differentiation (mean F_{ST} = 0.244) between fifteen Spanish chicken breeds using 24 microsatellite markers.

The pairwise F_{ST} computed between the breeds was generally high; however, two breeds (Orusthöna and Gammelsvensk Dvärghöna) did not show significant genetic differentiation with any of the studied breeds (Appendix 3 Table S8). This may be associated with the small sample sizes of breeds, because Orusthöna and Gammelsvensk Dvärghöna are among the breeds with the smallest sample sizes. Though statistical significance test based on P-value largely depend on sample sizes, F_{ST} has a strong association with genetic distance and commonly used as a measure of genetic relationship between populations (Takezeki and Nei, 2008). Thus, breeds which did not show significant genetic differentiation may contain admixed and migrant individuals which might share ancestors in more than one breed. The pairwise F_{ST} comparisons of Gotlandshöna, Hedemorahöna and Öländsk Dvärghöna breeds showed significant differentiations with all breeds except with Orusthöna and Gammelsvensk Dvärghöna breeds.

4.3. Genetic contribution of breeds

The genetic contribution of breeds to the global genetic diversity was assessed by accounting the within and between genetic diversity of breeds (Caballero and Toro, 2002). Accordingly, the highest loss of total genetic diversity (-3.407%) was incurred when the Gotlandshöna breed is disregarded (Table 4). This is because of loss of the within (-0.583%) and between (-2.824%) breed genetic diversity of the population resulting from extinction of Gotlandshöna breed. Relatively high within breed genetic diversity gains and at the same time loss of between breed genetic diversity was observed when the Svarthöna breed was ignored. This may be because the Svarthöna breed has low within breed genetic diversity (Table 2) estimated using Nei's (1987) within breed expected heterozygosity. As a result, low within breed heterozygosity favoured high genetic diversity between breeds (Ollivier and Foulley, 2005). Thus, removal of such breeds from the metapopulation could result in loss of genetic diversity between breeds. Similarly, exclusion of breeds showing low within breed genetic diversity would increase the within breed mean genetic diversity of the total population. Possibly due to similar reasons, removal of Ölandshöna, Gammelsvensk Dvärghöna and Orusthöna breeds showed genetic gain within breed but genetic losses between breeds. When the Skånsk blommehöna breed was taken out of the total population, loss of the within breed diversity and at the same time gain of the between breeds genetic diversity was observed

(Table 4). This is because the within breed heterozygosity of the Skånsk blommehöna breed was relatively high compared to other breeds such as Svarthöna.

All local Swedish chicken breeds included in the present study are maintained in a genetic conservation program undertaken by the Swedish association for local poultry (SLK) (http://www.kackel.se). The SLK was established in 1986 with the aim of preserving and propagating of native Swedish chickens and other poultry species such as Duck and Geese in the form of live gene bank through its members. Basically, implementing effective and sustainable genetic conservation program requires long term investment. Thus, the present study could help to prioritize breeds, for instance, in minimizing the within breed coancestry and controlling inbreeding, based on their genetic contributions to the global chicken genetic diversity. However, the present study is based on molecular information and the method proposed by Caballero and Toro (2002). Because applying other methods and considering non-genetic information may give different results.

4.4. Phylogenetic relationships

Based on Nei's genetic distance and neighbor-joining method, the ten local Swedish chicken breeds were broadly but weakly (bootstrap value = 7.5%) grouped into two clusters (Figure 1). One of the clusters contained Svarthöna, Gammelsvensk Dvärghöna, Orusthöna, Gotlandshöna and Ölandshöna as an out group breed. The second cluster contained the remaining five breeds. Bootstrap values that support the level of genetic relationship between breeds were generally low and ranged from 7.5% to 62.2% (Figure 1). This may be associated with small sample size and the occurrence of more number of monomorphic loci in some of the breeds included in this study. For example, Orusthöna breed contained only two individuals and half of the 24 loci were monomorphic in this breed. When breeds containing fewer than ten individuals (Orusthöna, Kindahöna, Gammelsvensk Dvärghöna, Ölandshöna and Åsbohöna) were excluded from the phylogeny, the statistical evidences which support the genetic relationship between breeds were considerably increased (Figure 2). Accordingly, Gotlandshöna and Svarthöna, and Hedemorahöna and Skånsk blommehöna have close genetic relationships which were also consistent with Figure 1 despite low bootstrap value. Takezaki and Nei (2008) studied the reliability of phylogenetic tree topology using microsatellite data and advised to use at least 30 microsatellite loci and fifteen individuals per population to infer more reliable genetic relationships. This is in agreement with Figure 2 of our result which showed high bootstrap values though we used 24 microsatellite loci and included two breeds containing ten and fourteen individuals.

The phylogenetic tree was reconstructed by splitting the ten breeds into seventeen flocks (Figure 3). The reason for subdividing breeds into flocks was to identify possible migrants and breed admixtures, because some breeds such as Gotlandshöna and Hedemorahöna have more than two flocks which were sampled from different sources. Consequently, the two flocks of Skånsk blommehöna breed were clustered differently but with low bootstrap confidences. Similarly, two of the Gotlandshöna flocks were assigned in the same tree branch in 91.3% of the time but the third flock of Gotlandshöna was assigned with other breed's flocks. Though assigning of some flocks outside their breed was not supported by strong

statistical evidences, this may indicate the presence of admixed and migrant individuals in some of local Swedish chicken breeds. The two Svarthöna flocks were assigned together with strong statistical evidence (bootstrap value = 99.9%).

4.5. Genetic structure of breeds

The genetic structure of breeds was studied using a model-based clustering approach which assigns individuals into one or more populations probabilistically based on the allele frequencies detected at different loci. According to Evanno et al. (2005), the most likely number of clusters of local Swedish chickens was found at K = 4 (Figure 4 and 5). Because the rate of changes in the log likelihood (Delta K) of the data was highest when individuals in all breeds were assigned into four clusters. At K = 4, individuals in the Gotlandshöna, Hedemorahöna, Öländsk Dvärghöna and Svarthöna breeds were assigned distinctively into their respective breeds but the remaining six breeds exhibited admixed mosaic clusters. This is in agreement with the results of the pairwise F_{ST} comparisons where some of the breeds did not show significant genetic differentiations, particularly the five breeds which contained very small number of individuals ranging from two to five (Åsbohöna, Kindahöna, Gammelsvensk Dvärghöna, Ölandshöna and Orusthöna). First, this may be associated with the effects of differences in sample size. Because breeds with relatively large sample size formed their own cluster, but those with small sample sizes failed to show genetic differentiation and hence, clustered together. Based on simulated data, Kalinowski (2011) showed that STRUCTURE based clustering of individuals could potentially be affected by variations in sample size. Second, chickens may have recent ancestors in more than one breed and as a result could be clustered in more than one breed (Pritchard et al., 2000). The presence of admixed individuals could also be the cause for low bootstrap values observed in the neighbor-joining tree (Figure 1) discussed earlier. Because neighbor joining tree deals with genetic distance between breeds and as a result the frequency of gene exchange between breeds, especially due to the presence of admixed individuals, could compromise the statistical power of the tree topology (Pritchard et al. 2000; Rosenberg et al. 2001).

At K = 5, individuals in Skånsk blommehöna, Åsbohöna, Kindahöna, Ölandshöna, Gammelsvensk Dvärghöna and Orusthöna breeds were uniformly clustered together unlike the mosaic clusters appeared at K = 4. In addition, the average similarity coefficient of individuals at K = 5 was larger than the corresponding estimate at K = 4 (98% vs 93%). However, based on Evanno et al. (2005) method, Delta K was slightly larger at K = 4 and as a result the most probable number of clusters of the ten breeds was taken as four. To clarify the ambiguity of breeds clustering at K = 4 and 5, we have performed a substructure analysis for eight breeds (excluding Gotlandshöna and Hedemorahöna). The reason for exclusion of these two breeds from the second structure analysis was that the clustering patterns of these two breeds were similar both at K = 4 and 5. Results of substructure analysis (Figure 6 and Table 6) showed that the most possible number of clusters for eight local Swedish chicken breeds was found at K = 3, which is completely in line with the first structure analysis found at K = 5 in Figure 4. This may indicate that the most probable number of clusters of the ten breeds may be either K =4 or 5 as shown in Figure 4, because according to Rosenberg et al. (2001) and Evanno et al. (2005), inferring the true value of K may depend on several factors,

for example sample size, type and number of loci, the level of genetic differentiation and marker polymorphism.

5. CONCLUSIONS

The present study is the first study to analyse the genetic diversity, genetic relationships and population structure of local Swedish chicken breeds using microsatellite data. The presence of high inbreeding within the majority of local Swedish chicken breeds lowered their genetic diversity but still there is at least moderate variation in genetic diversity between breeds. Regardless of the methods applied to assess genetic differentiations between breeds (i.e. Fixation indices, neighbor-joining and structure clustering), four of the ten breeds (Gotlandshöna, Hedemorahöna, Öländsk Dvärghöna and Svarthöna) were genetically distinct except few migrant individuals observed in Gotlandshöna. The other six breeds (Skånsk blommehöna, Åsbohöna, Kindahöna, Ölandshöna, Gammelsvensk Dvärghöna and Orusthöna) did not show significant genetic differentiations and this was mainly due to small sample sizes. This study also demonstrated the possibility of getting more information on genetic diversity of chickens using microsatellite loci even when sample sizes are too few. The results of the current study can be used as baseline genetic information that can be assimilated with genetic conservation programs, for instance, to control inbreeding within breeds and to implement further genetic studies in local Swedish chickens. However, it is difficult to expect more reliable results from such a small data set. Therefore, it is important to study the genetic diversity of local Swedish chickens using sufficient sample sizes.

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7. REFERENCES

Akishinonomiya, F., Miyake, T., Sumi, S., Takada, M., Ohno, S. and Kondo, N. 1994. One subspecies of the red junglefowl (Gallus gallus gallus) suffices as the matriarchic ancestor of all domestic breeds. Proc. Natl. Acad. Sci. USA, 91:12505-12509.

Akishinonomiya, F., Miyake, T., Takada, M., Shingu, R., Endo, T., et al. 1996. Monophyletic origin and unique dispersal patterns of domestic fowls. Proc Natl Acad Sci USA, 93: 6792-6795.

Anmarkrud, J.A., Kleven, O., Bachmann, L. and Lifjeld, J. T. 2008. Microsatellite evolution: Mutations, sequence variation, and homoplasy in the hypervariable avian microsatellite locus HrU10. BMC Evolutionary Biology, 8:138-1347.

Blackburn, H. D. 2006. The National Animal Germplasm Program: Challenges and Opportunities for Poultry Genetic Resources. Poultry Science, 85:210-215.

Caballero, A. and Toro, M. A. 2002. Analysis of genetic diversity for the management of conserved subdivided populations. Conservation Genetics, 3:289-299.

Castro, J., Bouza, C., Presa, P., Pino-Querido, A., Riaza, A., Ferreiro, I., Sánchez, L. and Martínez, P. 2004. Potential sources of error in parentage assessment of turbot (Scophthalmus maximus) using microsatellite loci. Aquaculture, 242:119-135.

Chen, G., Bao, W., Shu, J., Ji, C., Wang, M., Eding, H., Muchadeyi, F. and Weigend, S. 2008. Assessment of Population Structure and Genetic Diversity of 15 Chinese Indigenous Chicken Breeds Using Microsatellite Markers. Asian-Aust. J. Anim. Sci., 21(3): 331-339.

Crawford, R.D. 1990. Origin and history of poultry species. In: R.D. Crawford (Ed.), Poultry Breeding and Genetics, Elsevier, Amsterdam, pp. 1-42.

Cuc , N. T. K., Simianer, H., Eding ,H., Tieu, H. V., Cuong, V. C., Wollny, C. B. A., Groeneveld, L. F. and Weigend, S. 2010. Assessing genetic diversity of Vietnamese local chicken breeds using microsatellites. Animal Genetics, 41, 545-547.

Dakin, E. and Avise, J. C. 2004. Microsatellite null alleles in parentage analysis. Heredity, 93:504-509.

Dávila, S. G., Gil, M. G., Resino-Talavan, P. and Campo, J. L. 2009. Evaluation of diversity between different Spanish chicken breeds, a tester line, and a White Leghorn population based on microsatellite markers. Poultry Science, 88:2518-2525.

Dessie, T., Dana, N., Ayalew, W. and Hanotte, O. 2012. Current state of knowledge on indigenous chicken genetic resources of the tropics: domestication, distribution and documentation of information on the genetic resources. World's Poultry Science Journal, 68:11-20.

Dorshorst, B., Molin, A-M., Rubin, C.J., Johansson, A.M., Strömstedt, L., et al. 2011. A Complex Genomic Rearrangement Involving the Endothelin 3 Locus Causes Dermal Hyperpigmentation in the Chicken. PLoS Genet, 7(12): e1002412. doi:10,1371/journal.pgen.1002412.

Earl, D. A. and vonHoldt, B.M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4 (2):359-361.

Eltanany, M., Philipp, U., Weigend, S. and Distl, O. 2010. Genetic diversity of ten Egyptian chicken strains using 29 microsatellite markers. Animal Genetics, 42: 666-669.

Eriksson, J., Larson, G., Gunnarsson, U., Bed'hom, B., Tixier-Boichard, M., et al. 2008. Identification of the Yellow Skin Gene Reveals a Hybrid Origin of the Domestic Chicken. PLoS Genet, 4(2): e1000010. doi:10.1371/journal.pgen.1000010.

Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14: 2611-2620.

FAO (Food and Agriculture Organization). 2007. The State of the World's Animal Genetic Resources for Food and Agriculture. Edi: Barbara Rischkowsky & Dafydd Pilling. Rome. Italy.

FAO (Food and Agriculture Organization). 2011. Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No. 9. Rome. Italy.

Felsenstein, J. 2005. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle. Available at: http://evolution.genetics.washington.edu/phylip/getme.html [Accessed on 24/04/2013].

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available at: http://www.unil.ch/izea/softwares/fstat.html [Accessed on 20/04/2013].

Granevitze, Z., Hillel, J., Chen, G. H., Cuc, N. T. K., Feldman, M., Eding, H. and Weigend, S. 2007. Genetic diversity within chicken populations from different continents and management histories. Animal Genetics, 38:576-583.

Guo, X. and Elston, R.C. 1999. Linkage informative content of polymorphic genetic Markers. Hum. Hered., 49:112-118.

Gutiérrez, J.P., Royo, L.J., Álvarez, I., Goyache, F. 2005. MolKin v2.0: a computer program for genetic analysis of populations using molecular coancestry information. Journal of Heredity, 96: 718-721.

Hillel, J., Granevitze, Z., Twito, T., Ben-Avraham, D., Blum, S., Lavi, U., David, L., Feldman, M.W., Cheng, H. and Weigend, S. 2007. Molecular markers for the assessment of chicken biodiversity. World's Poultry Science Journal, 63: 33-45.

Hillel, J., Groenen, A. M. M., Tixier-Boichard, M., Korol, A. B., David, L., Kirzhner, V. M., Burke, T., Barre-Dirie, A., Crooijmans, R. P. M. A., Elo, K., Feldman, M. W., Freidlin, P. J., Mäki-Tanila, A., Oortwijn, M., Thomson, P., Vignal, A., Wimmers, K. and Weigend, S. 2003. Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. Genet. Sel. Evol., 35: 533-557.

Hubisz, M. J., Falush, D., Stephens, M. and Pritchard, J.K. 2009. Technical Advances. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources, 9:1322-1332.

Jakobsson, M. and Rosenberg, N. A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics, 23: 1801-1806.

Kalinowski, S.T., Taper, M.L. and Marshall, T.C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol., 16:1099-1106.

Kalinowski, S.T. 2011. The computer program STRUCTURE does not reliably identify the main genetic clusters within species: simulations and implications for human population structure. Heredity, 106(4): 625–632.

Kaya, M. and Yildiz, M.A. 2008. Genetic Diversity among Turkish Native Chickens, Denizli and Gerze, Estimated by Microsatellite Markers. Biochem Genet, 46:480-491.

Li, Y.C., Korol, A.B., Fahima, T., Beiles, A. and Nevo, E. 2002. Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Molecular Ecology, 11: 2453-2465.

Moiseyeva, I.G., Romanov, M.N., Nikiforov, A.A., Sevastyanova, A.A. and Semyenova, S.K. 2002. Evolutionary relationships of Red Jungle Fowl and chicken breeds. Genet. Sel. Evol., 35:403-423.

Muchadeyi, F.C., Eding, H., Wollny, C.B.A., Groeneveld, E., Makuza, S.M., Shamseldin, R., Simianer, H. and Weigend, S. 2007. Absence of population sub-structuring in Zimbabwe chicken ecotypes inferred using microsatellite analysis. Animal Genetics, 38:332-339.

Nei, M. 1972. Genetic Distance between Populations. The American Naturalist, 106:283-292.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Niu, D., Fu, Y., Luo, J., Ruan, H., Yu, X.P., Chen, G. and Zhang, Y.P. 2002. The origin and genetic diversity of Chinese Native Chicken breeds. Biochemical Genetics, 40:163-174.

Ollivier, L. and Foulley, J.L. 2005. Aggregate diversity: New approach combining within- and between-breed genetic diversity. Livestock Production Science, 95(3):247-254.

Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multi-locus genotype data. Genetics, 155:945–959.

Rajkumar, U., Gupta, B.R., Ahmed, N., Venktramaiah, A. and Reddy, A.R. 2007. Genetic variation and genetic diversity in chicken populations using microsatellite assay. Indian Journal of Animal Sciences, 77(11):1194-1198.

Ramadan, S., Kayang, B.B., Inoue, E., Nirasawa, K., Hayakawa, H., Ito, S. and Inoue-Murayama, M. 2012. Evaluation of genetic diversity and conservation priorities for Egyptian chickens. Journal of animal science, 2(3):183-190.

Romanov, M. N. and Weigend, S. 2001. Analysis of Genetic Relationships between Various Populations of Domestic and Jungle Fowl Using Microsatellite Markers. Poultry Science, 80:1057-1063.

Rosenberg, N.A., Burke, T., Elo, K., Feldman, M.W., Freidlin, P.J., Groenen, M.A., Hillel, J., Mäki-Tanila, A., Tixier-Boichard, M., Vignal, A., Wimmers, K. and Weigend, S. 2001. Empirical evaluation of genetic clustering methods using multi-locus genotypes from 20 chicken breeds. Genetics, 159(2):699-713.

Rosenberg, NA. 2004. Distruct: a program for the graphical display of population structure. Molecular Ecology Notes, 4:137-138.

Storey, A.A., Athens, J.S., Bryant, D., Carson, M., Emery, K., et al. 2012. Investigating the Global Dispersal of Chickens in Prehistory Using Ancient Mitochondrial DNA Signatures. PLoS ONE 7(7): e39171. doi:10.1371/journal.pone.0039171.

Tadano, R., Nishibori, M., Imamura, Y., Matsuzaki, M., Kinoshita, K., Mizutani, M., Namikawa, T. and Tsudzuki, M. 2007. High genetic divergence in miniature breeds of Japanese native chickens compared to Red Junglefowl, as revealed by microsatellite analysis. Animal Genetics, 39: 71-78.

Takezaki, N. and Nei, M. 2008. Empirical Tests of the Reliability of Phylogenetic Trees Constructed With Microsatellite DNA. Genetics, 178: 385–392.

Vanhala, T., Tuiskula-Haavisto, M., Elo, K., Vilkki, J. and Mäki-Tanila A. 1998. Evaluation of genetic variability and genetic distances between eight chicken lines using microsatellite markers. Poultry Science, 77(6):783-90.

Weir, B. S. and Cockerham, C. C. 1984. Estimating F-Statistics for the Analysis of Population Structure. Evolution, 38 (6): 1358-1370.

West, B. and Zhou, B.X. 1988. Did chicken go north? New evidence for domestication. Journal of Archaeological Science, 15: 515-533.

Wilkinson, S., Wiener, P., Teverson, D., Haleyand, C. S. and Hocking, P. M. 2011. Characterization of the genetic diversity, structure and admixture of British chicken breeds. Animal Genetics, 43:552-563.

Young A.G. and Clarke G.M. (Eds). 2000. Genetics, Demography and Viability of Fragmented Populations. Cambridge. UK: Cambridge University press.

Zanetti, E., De Marchi, M., Dalvit, C. and Cassandro, M. 2010. Genetic characterization of local Italian breeds of chickens undergoing in situ conservation. Poultry Science, 89:420-427.

Appendix 1 Microsatellite loci used to genotype local Swedish chickens

Table S1. Name of loci, chromosome number, primer sequences (forward and reverse primer), Gene bank accession number and multiplex PCR group of microsatellite loci used to genotype individuals sampled from ten local Swedish Chicken breeds. All loci are member of the 30 microsatellite loci recommended by ISAG-FAO (FAO, 2011)

Loci	Chromosome number	Primer sequences (Forward and reverse primers) (5' –> 3')	Gene bank accession	Multiplex group
LEI0094	4	GATCTCACCAGTATGAGCTGC TCTCACACTGTAACACAGTGC	X83246	1
ADL0268	1	CTCCACCCCTCTCAGAACTA CAACTTCCCATCTACCTACT	G01688	1
MCW0248	1	GTTGTTCAAAAGAAGATGCATG TTGCATTAACTGGGCACTTTC	G32016	1
MCW0216	13	GGGTTTTACAGGATGGGACG AGTTTCACTCCCAGGGCTCG	AF030586	1
MCW0295	4	ATCACTACAGAACACCCTCTC TATGTATGCACGCAGATATCC	G32052	1
MCW0081	5	GTTGCTGAGAGCCTGGTGCAG CCTGTATGTGGAATTACTTCTC	L43636	2
MCW0069	26	GCACTCGAGAAAACTTCCTGCG ATTGCTTCAGCAAGCATGGGAGGA	L43684	2
MCW0034	2	TGCACGCACTTACATACTTAGAGA TGTCCTTCCAATTACATTCATGGG	L43674	2
MCW0222	3	GCAGTTACATTGAAATGATTCC TTCTCAAAACACCTAGAAGAC	G31996	2
MCW0111	1	GCTCCATGTGAAGTGGTTTA ATGTCCACTTGTCAATGATG	L48909	2
MCW0037	3	ACCGGTGCCATCAATTACCTATTA GAAAGCTCACATGACACTGCGAAA	L43676	3
LEI0166	3	CTCCTGCCCTTAGCTACGCA TATCCCCTGGCTGGGAGTTT	X85531	3

Table S1. Continued

Loci	Chromosome number	Primer sequences (Forward and reverse primers) (5' –> 3')	Gene bank accession	Multiplex group
ADL112	10	GGCTTAAGCTGACCCATTAT ATCTCAAATGTAATGCGTGC	G01725	3
MCW0014	6	TATTGGCTCTAGGAACTGTC GAAATGAAGGTAAGACTAGC	L40040	4
MCW0183	7	ATCCCAGTGTCGAGTATCCGA TGAGATTTACTGGAGCCTGCC	G31974	4
ADL0278	8	CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAACAGTGTG	G01698	4
MCW0123	14	CCACTAGAAAAGAACATCCTC GGCTGATGTAAGAAGGGATGA	L43645	5
MCW0165	23	CAGACATGCATGCCCAGATGA GATCCAGTCCTGCAGGCTGC	L43663	5
MCW0020	1	TCTTCTTTGACATGAATTGGCA GCAAGGAAGATTTTGTACAAAATC	L40055	5
MCW0104	13	TAGCACAACTCAAGCTGTGAG AGACTTGCACAGCTGTGTACC	L43640	5
MCW0078	5	CCACACGGAGAGGAGAAGGTCT TAGCATATGAGTGTACTGAGCTTC	L43686	6
MCW0067	10	GCACTACTGTGTGCTGCAGTTT GAGATGTAGTTGCCACATTCCGAC	G31945	6
MCW0330	17	TGGACCTCATCAGTCTGACAG AATGTTCTCATAGAGTTCCTGC	G32085	6
MCW0098	4	GGCTGCTTTGTGCTCTTCTCG CGATGGTCGTAATTCTCACGT	L40074	6

Appendix 2 Polymerase Chain Reaction (PCR) protocols

Table S2. PCR protocols for multiplex 1(on the left side of the table and 2 (on the right side of the table) containing the QIAGEN multiplex PCR buffer with Magnesium Chloride (MgCl2), deoxynucleotide triphosphates (dNTPs), HotStar Taq DNA polymerase enzyme, distilled water, fluorescently labeled forward and unlabeled reverse primers of the associated locus, the genomic DNA and a 1X reaction volume required from each PCR reagent

multiplex 1		multiplex 2		
PCR reagents	volume (µl) 1X	PCR reagents	volume (µl)	1X
10xbuff incl $MgCl_2$	1.000	10xbuff incl $MgCl_2$	1.000	
dNTPs (25 mM)	0.080	dNTPs (25 mM)	0.080	
QIAGEN HotStar Taq (5U/ µl)	0.100	QIAGEN HotStar Taq (5U/ µl)	0.100	
H2O	7.160	H2O	7.870	
LEI0094_F (10 µM)	0.250	MCW0295_F (10 µM)	0.025	
LEI0094_R (10 µM)	0.250	MCW0295_R (10 µM)	0.025	
ADL0268_F (10 µM)	0.050	MCW0081_F (10 µM)	0.025	
ADL0268_R (10 µM)	0.050	MCW0081_R (10 µM)	0.025	
MCW0248_F (10 µM)	0.200	MCW0069_F (10 µM)	0.025	
MCW0248_R (10 µM)	0.200	MCW0069_R (10 µM)	0.025	
MCW0216_F (10 µM)	0.030	MCW0034_F (10 µM)	0.100	
MCW0216_R (10 µM)	0.030	MCW0034_R (10 µM)	0.100	
ADL0278_F (10 µM)	0.050	MCW0222_F (10 µM)	0.050	
ADL0278_R (10 µM)	0.050	MCW0222_R (10 µM)	0.050	
DNA	0.500	DNA	0.500	
total	10.00	total	10.00	

Table S3. PCR protocols for multiplex 3(on the left side of the table and 4 (on the right side of the table) containing the QIAGEN multiplex PCR buffer with Magnesium Chloride (MgCl2), deoxynucleotide triphosphates (dNTPs), HotStar Taq DNA polymerase enzyme, distilled water, fluorescently labeled forward and unlabeled reverse primers of the associated locus, the genomic DNA and a 1X reaction volume required from each PCR reagent

multiplex 3		multiplex 4	
PCR reagents	volume (µl) 1X	PCR reagents	volume (µl) 1X
10xbuff incl MgCl2	1.000	10xbuff incl MgCl2	1.000
dNTPs (25 mM)	0.080	dNTPs (25 mM)	0.080
QIAGEN HotStar Taq (5U/ µl)	0.100	QIAGEN HotStar Taq (5U/ µl)	0.100
H2O	7.414	H2O	8.18
MCW0111_F (10 µM)	0.003	ADL0112_F (10 µM)	0.010
MCW0111_R (10 µM)	0.003	ADL0112_R (10 µM)	0.010
MCW0037_F (10 µM)	0.050	MCW0014_F (10 µM)	0.010
MCW0037_R (10 µM)	0.050	MCW0014_R (10 µM)	0.010
LEI0166_F (10 µM)	0.200	MCW0183_F (10 µM)	0.050
LEI0166_R (10 µM)	0.200	MCW0183_R (10 µM)	0.050
DNA	0.500	DNA	0.500
total	10.00	total	10.00

Table S4. PCR protocols for multiplex 5(on the left side of the table and 6 (on the right side of the table) containing the QIAGEN multiplex PCR buffer with Magnesium Chloride (MgCl2), deoxynucleotide triphosphates (dNTPs), HotStar Taq DNA polymerase enzyme, distilled water, fluorescently labeled forward and unlabeled reverse primers of the associated locus, the genomic DNA and a 1X reaction volume required from each PCR reagent

multiplex 5		multiplex 6	
PCR reagents	volume (µl) 1X	PCR reagents	volume (µl) 1X
10xbuff incl MgCl2	1.000	10xbuff incl MgCl2	1.000
dNTPs (25 mM)	0.080	dNTPs (25 mM)	0.080
QIAGEN HotStarTaq (5U/µl)	0.100	QIAGEN HotStarTaq (5U/µl)	0.100
H2O	7.92	H2O	7.62
MCW0123_F (10 µM)	0.050	MCW0078_F (10 µM)	0.050
MCW0123_R (10 µM)	0.050	MCW0078_R (10 µM)	0.050
MCW0165_F (10 µM)	0.050	MCW0067_F (10 µM)	0.100
MCW0165_R (10 µM)	0.050	MCW0067_R (10 µM)	0.100
MCW0020_F (10 µM)	0.050	MCW0330_F (10 µM)	0.100
MCW0020_R (10 µM)	0.050	MCW0330_R (10 µM)	0.100
MCW0104_F (10 µM)	0.050	MCW0098_F (10 µM)	0.100
MCW0104_R (10 µM)	0.050	MCW0098_R (10 µM)	0.100
DNA	0.500	DNA	0.500
total	10	total	10

Table S5. Temperature setup and number of cycles for all PCR multiplexes (1-6)

Stages of PCR amplification	Temperature (ºC)	period	No. cycle
Initial incubation and enzyme activation	95 ºC	5 min	-
Denaturation	90 ºC	30 sec	
Primer annealing	55 ºC	45 sec	x 35
Extension	72 ºC	30 sec	
Final extension	72 ºC	15 min	-

Appendix 3 Genetic diversity and breed differentiation within and between ten local Swedish chicken breeds

Table S6. Observed heterozygosity per locus and breed of local Swedish chickens

Locus	Breeds*									
	Gotl	Hedem	Ölandsk	Skånsk	Ölands	Åsbo	Kinda	Gammel	Svart	Orust
LEI0094	0.848	0.111	0.294	0.200	0.000	0.800	0.000	0.333	0.000	0.500
ADL0268	0.394	0.139	0.588	0.600	0.250	0.200	0.000	0.667	0.000	0.000
MCW0248	0.061	0.444	0.412	0.100	0.250	0.200	1.000	0.000	0.000	0.000
MCW0216	0.212	0.194	0.412	0.400	0.500	0.800	0.000	0.000	0.286	0.000
ADL0278	0.606	0.000	0.588	0.500	0.000	1.000	0.333	0.667	0.500	0.000
MCW0295	0.303	0.583	0.412	0.200	0.000	0.000	0.000	0.333	0.000	0.500
MCW0081	0.364	0.306	0.529	0.600	0.000	0.800	0.000	1.000	0.429	0.500
MCW0069	0.394	0.306	0.235	0.400	0.000	0.400	0.667	0.000	0.462	0.500
MCW0034	0.303	0.639	0.706	0.400	0.500	0.600	0.000	0.667	0.357	0.000
MCW0222	0.242	0.111	0.353	0.800	0.000	0.000	0.667	0.667	0.000	0.000
MCW0111	0.121	0.417	0.000	0.600	0.000	0.400	0.667	0.000	0.357	0.000
MCW0037	0.939	0.750	0.647	0.900	0.500	1.000	1.000	0.667	0.500	1.000
LEI0166	0.152	0.389	0.471	0.600	0.500	0.800	0.333	0.667	0.643	1.000
ADL0112	0.242	0.389	0.353	0.500	0.500	0.000	0.000	0.000	0.000	0.500
MCW0014	0.000	0.139	0.000	0.100	0.000	0.200	0.333	0.500	0.000	0.000
MCW0183	0.485	0.167	0.000	0.300	0.250	0.000	0.333	0.333	0.000	1.000
MCW0123	0.515	0.278	0.000	0.200	0.000	0.200	0.333	0.000	0.500	0.000
MCW0165	0.061	0.222	0.118	0.000	0.000	0.000	0.333	0.000	0.000	0.000
MCW0020	0.545	0.306	0.353	0.700	0.500	0.600	1.000	0.333	0.071	0.500

Table S6. Continued

Locus	Breeds*									
	Gotl	Hedem	Ölandsk	Skånsk	Ölands	Åsbo	Kinda	Gammel	Svart	Orust
MCW0104	0.061	0.028	0.375	0.400	0.750	0.200	0.667	0.000	0.143	0.000
MCW0078	0.030	0.667	0.176	0.600	0.000	0.800	1.000	1.000	0.000	0.500
MCW0067	0.061	0.111	0.353	0.200	0.250	0.200	0.333	0.667	0.571	0.000
MCW0330	0.121	0.250	0.000	0.500	0.250	0.400	0.333	0.000	0.571	0.000
MCW0098	0.576	0.389	0.353	0.000	0.250	0.400	0.333	0.667	0.000	0.000

*Gotl = Gotlandshöna; Hedem = Hedemorahöna; Ölandsk = Ölandsk Dvärghöna; Skånsk = Skånsk blommehöna; Ölands = Ölandshöna; Åsbo = Åsbohöna; Kinda = Kindahöna; Gammel = Gammelsvensk Dvärghöna; Svart = Svarthöna; Orust = Orusthöna

Table S7. Nei's (1987) unbiased gene diversity (expected heterozygosity) per locus and breed of local Swedish chickens

	Breeds*									
loci	Gotl	Hedem	Ölandsk	Skånsk	Ölands	Åsbo	Kinda	Gammel	Svart	Orust
LEI0094	0.755	0.284	0.493	0.483	0.000	0.575	0.000	0.333	0.000	0.500
ADL0268	0.511	0.337	0.542	0.572	0.500	0.600	0.667	0.500	0.000	1.000
MCW0248	0.224	0.491	0.397	0.411	0.250	0.200	0.667	0.000	0.000	0.000
MCW0216	0.333	0.416	0.401	0.333	0.583	0.575	0.667	0.000	0.352	0.000
ADL0278	0.693	0.000	0.535	0.561	0.000	0.600	0.833	0.833	0.473	0.000
MCW0295	0.356	0.713	0.691	0.556	0.000	0.600	0.000	0.667	0.318	0.500
MCW0081	0.428	0.562	0.485	0.678	0.000	0.725	0.000	0.750	0.346	0.500
MCW0069	0.417	0.615	0.507	0.711	0.000	0.350	0.833	0.000	0.365	0.500
MCW0034	0.500	0.549	0.608	0.656	0.750	0.500	0.667	0.500	0.407	0.000
MCW0222	0.216	0.106	0.447	0.528	0.000	0.000	0.500	0.500	0.000	0.000
MCW0111	0.169	0.508	0.000	0.744	0.000	0.550	0.500	0.000	0.302	0.000
MCW0037	0.545	0.587	0.511	0.656	0.708	0.750	0.500	0.500	0.489	0.500
LEI0166	0.145	0.509	0.426	0.561	0.625	0.575	0.333	0.500	0.646	0.750

Table S7 continued

	Breeds*									
loci	Gotl	Hedem	Ölandsk	Skånsk	Ölands	Åsbo	Kinda	Gammel	Svart	Orust
ADL0112	0.291	0.431	0.371	0.567	0.417	0.000	0.667	0.000	0.000	0.500
MCW0014	0.515	0.617	0.000	0.100	0.000	0.500	0.667	1.000	0.000	1.000
MCW0183	0.621	0.252	0.000	0.644	0.250	0.000	0.667	0.333	0.143	0.500
MCW0123	0.477	0.471	0.000	0.194	0.000	0.750	0.667	0.000	0.577	0.000
MCW0165	0.173	0.205	0.526	0.600	0.000	0.700	0.667	0.000	0.000	0.000
MCW0020	0.642	0.399	0.474	0.722	0.417	0.700	0.833	0.333	0.071	0.500
MCW0104	0.060	0.056	0.313	0.639	0.500	0.650	0.500	0.667	0.137	0.000
MCW0078	0.030	0.490	0.261	0.500	0.000	0.500	0.750	0.500	0.000	0.500
MCW0067	0.116	0.297	0.371	0.561	0.250	0.200	0.667	0.500	0.418	0.000
MCW0330	0.527	0.229	0.515	0.522	0.583	0.375	0.833	0.000	0.505	0.000
MCW0098	0.476	0.509	0.298	0.000	0.583	0.550	0.333	0.500	0.000	0.000

*Gotl = Gotlandshöna; Hedem = Hedemorahöna; Ölandsk = Ölandsk Dvärghöna; Skånsk = Skånsk blommehöna; Ölands = Ölandshöna; Åsbo = Åsbohöna; Kinda = Kindahöna; Gammel = Gammelsvensk Dvärghöna; Svart = Svarthöna; Orust = Orusthöna

Table S8. Pairwise F_{ST} (Weir and Cockerham, 1984) estimates between ten local Swedish chicken breeds (below diagonal) and the level of significance of the associated pairs calculated following Bonferroni corrections for multiple comparisons (above diagonal)

No.	breeds	1	2	3	4	5	6	7	8	9	10
1	Gotlandshöna		***[a]	***	***	***	***	**[b]	NS[d]	***	NS
2	Hedemorahöna	0.457		***	***	***	***	*[c]	NS	***	NS
3	Öländsk Dvärghöna	0.472	0.388		***	*	**	*	NS	***	NS
4	Skånsk blommehöna	0.296	0.314	0.325		*	*	NS	NS	***	NS
5	Ölandshöna	0.368	0.448	0.487	0.341		NS	NS	NS	*	NS
6	Åsbohöna	0.384	0.359	0.338	0.208	0.387		NS	NS	**	NS
7	Kindahöna	0.417	0.312	0.405	0.230	0.356	0.269		NS	NS	NS
8	Gammelsvensk Dvärghöna	0.389	0.355	0.414	0.232	0.463	0.310	0.306		NS	NS
9	Svarthöna	0.498	0.512	0.551	0.424	0.614	0.453	0.510	0.508		NS
10	Orusthöna	0.417	0.472	0.453	0.298	0.537	0.327	0.300	0.442	0.572	

[a] *$P \leq 0.001$ = ****

[b] *$P \leq 0.01$ = ***

[c] *$P \leq 0.05$ = **

[d] *NS = not significant*