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An Assessment of Genetic Diversity of Vietnamese H'mong Chickens

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Abstract: The objective of the study was to assess genetic diversity of H'mong chickens, a local breed in the mountainous areas of Northern Vietnam. Structured questionnaires were administered to fifty-five households from three villages (Phieng Cam (n = 30), Chieng Chan (n = 15) and Chieng Noi (n = 10). Morphological characters of 773 chickens were physically examined. Flock sizes averaged 14.44 ± 7.38 chickens per household. Seventy percent of the chickens had predominantly brown and multicoloured plumage. Yellow skin (94%) dominated over black skin colour. Ninety-five percent of the chickens had black legs. Ninety-six percent had black versus yellow beaks. Single comb prevailed with a frequency of 94%. Body weight of adult chickens averaged 1617g (± 52). Hens laid 12–13 eggs per clutch, with an average egg weight of 41 g. Hatching rate was more than 80%. The chickens were reared under a semi-scavenging production system in which 85% of the households provided chicken housing. All farmers supplemented their chickens with whole maize at most twice per day. A subset of thirty-six chickens from the three villages was genotyped at 29 microsatellite loci. A total of 186 alleles were observed. The mean number of alleles was 6.41 per locus. Heterozygosity varied from 62.7% to 66.8% for the three populations. All the village based populations were in Hardy-Weinberg equilibrium and were not affected by inbreeding. Pair wise F_{ST} indicated a significant ($P<0.05$) differentiation between the Chieng Chan and the other two populations. The Nei's, Reynold's and Cavalli-Sforza distance measures showed Chieng Chang to be more distant from the two geographically close populations. There was no significant ($P>0.05$) genetic differences among the plumage colour based populations. The highest number of identical structure runs (10 out of 100) were observed at K = 2 in which Phieng Cam and Chieng Noi chicken clustered as one population while the Chieng Chan population had some individuals partly assigned to the Phieng Cam and Chieng Noi cluster.

Key words: H'mong chickens, production system, microsatellites, genetic diversity

Introduction

H'mong chickens originated and are distributed in the mountainous areas of Northern Vietnam. These chickens are reared by the H'mong minority tribes in the least favourable and inaccessible land characterized by high altitude. It is assumed that the H'mong chickens are adapted to the local low input - low output production system (Hoang Van Tieu and Vo Van Su, 2000; Vo Van Su *et al*., 2001). They produce meat, which is considered tastier compared to that from other local chickens. The black meat type associated with some of these chicken populations is used as traditional medicine to improve human health (Hoang Van Tieu and Vo Van Su, 2000). Interest in H'mong chickens as an animal genetic resource is recent and goes back to 1996 (Phuong Thao and Mai Hoang, 2003; Hoang Van Tieu *et al*., 2001).

Phenotypic diversity of H'mong chickens is manifested in three feather colour variants (Hoang Van Tieu *et al*., 2001). These are the black, white and brown feathered groups. It is not known, however, whether the three feather colour variants are a true representation of genetically distinct subpopulations within the H'mong chickens. Furthermore, these chickens are reared by different communities in isolated villages. It needs to be investigated for the development of effective conservation programs, whether the chicken populations in different areas represent unique populations. An assessment of the genetic structure utilizing molecular tools such as microsatellite markers (Crooijmans *et al*., 1996; Vanhala *et al*., 1998; Zhou and Lamont, 1999; Marle-Köster and Nel, 2000, Wimmers *et al*., 2000; Romanov and Weigend, 2001) provides insight into the diversity within and between these indigenous chicken populations. An understanding of the production systems under which these chickens are reared helps in defining conservation units. The objectives of this study were therefore to describe the production system and assess genetic diversity of H'mong chicken populations located in three different villages.

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Materials and Methods

The study area and sampling procedure: The study was carried out in Maison District of Sonla Province in Northwest Vietnam. The province is located within latitudes 20°39´ and 22°00´ North and longitudes 103°11´ and 103°35´. East and represents the fifth largest province in the country. The altitude ranges from 600m to 700m above sea level. Average temperature ranged from 14.5°C to 24°C. The annual rainfall is 1414mm and the average relative humidity is about 80 %. The climate is divided into 2 distinct seasons: the rainy season from May to September and the dry season from October to April (Sonla Province Statistics Department, 2005).

The 1410km^2 district consists of 21 villages that are divided into three economic zones and rear approximately 360 000 chickens. Annual cash income per capita is 160, 140 and 75 USD in zone I, zone II, and zone III, respectively. H'mong chickens are kept in six of these villages.

Fifty-five households known to keep H'mong chickens were randomly selected from three villages of Chieng Chan (n = 15), Phieng Cam (n = 30) and Chieng Noi (n = 10). Phieng Cam and Chieng Noi are neighboring villages. The distance to Chieng Chan is directly 20km but 100km by road. The H'mong chickens kept by these households are grouped into 3 categories based on plumage colour. The black-feathered chickens have a uniform black plumage colour while the white-feathered chickens have a uniform white feather plumage. The brown-feathered chickens refer to those chickens with brown and mixed plumage colour. From the three villages, 773 chickens of mixed age groups were randomly selected (Table 1).

Questionnaire administration and phenotypic characterization: A structured questionnaire was administered to the 55 households with the help of local H'mong interpreters. Data captured in the questionnaires included chicken management practices such as housing, feeding and marketing. Production data such as age at start of laying, clutch size and hatchability were recorded.

Each of the 773 chickens was physically examined. Plumage, skin, leg and comb colour were recorded as well as comb shape, live and egg weights. Both survey and phenotypic characterization were conducted between November 2003 and February 2004.

Blood sampling and DNA isolation: A total of 36 birds of three feather colour variants were sampled from 3 villages in which 6 samples for each sex per village and feather colour were used. One male and one female bird were sampled from a single household for each colour. From these chickens, a drop of venous blood was collected from the ulnar vein onto FTA@Micro Card (Whatman Bio Science, UK). The filter paper was allowed to dry, sealed in aluminum bags and kept at room temperature, awaiting DNA isolation. DNA isolation was done using a Phenol/Chloroform extraction method.

DNA polymorphism: The DNA polymorphism was determined using a set of 29 microsatellite markers. These markers are similar to the ones used in the AVIANDIV (1998-200) project and were selected based on their wide distribution over the genome (Table 2). The marker loci correspond to the revised set of microsatellites which were suggested by FAO (1995) for the MoDAD project (http://dad.fao.org/en/refer/library /guidelin/marker.pdf).

Polymerase chain reaction (PCR) was used to amplify the specific DNA fragments containing microsatellites. Two to five pairs of primers were run in one tube. Twenty nanograms of DNA, 10pmol of forward primer labeled with either IRD700 or IRD800 (MWG-Biotech, Ebersberg, Germany), 10 pmol of unlabeled reverse primer, and 1 mM tetramethylammonium chloride were mixed in the PCR tube. The amplification protocol involved initial denaturation of DNA and enzyme activation, at 95° (15min) followed by 35 cycles of denaturation at 95° (1min), primer annealing at temperature varying between 58° and 63° (1min), extension at 72° (1min), and final extension at 72° (10 min) using an automated thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany). DNA fragments produced by amplification were visualized as bands on 8% polyacrylamide gel, which was performed with a LI-COR semi-automated DNA analyzer (LI-COR Biotechnology Division, Lincoln, NE68504). Electrophoregram processing and allele-size scoring were performed with the RFLPscan package (Scanalytics, Division of CSP, Billerica, U.S.A.).

Statistical analysis

Chicken management practices and production performance: Descriptive statistics (SAS, 1999) were used to analyze for the chicken management practices. The FREQ procedure (SAS, 1999) was used to analyze for the feather, skin and leg colours as well as comb shape. Chicken live and egg weight were analyzed using the MEANS procedure of SAS (1999).

Genetic variation within populations: The observed number of alleles at each locus in each sample and the respective allele frequency were calculated using the FSTAT software, version 2.9.3 (Goudet, 2001).

Mean observed and expected frequency of heterozygoses for each population, and overall, as well F_{IS} and F_{IT} values for each locus and sample were calculated with microsatellite toolkit and FSTAT software. A chi–square test for Hardy–Weinberg equilibrium for each locus and overall was done using Genepop version 3.4 (Raymond and Rousset, 2004).

Table 1: Frequency (%) of feather, skin, leg and beak color in chickens collected in three villages

Location	n	Feather colour			Skin colour		Leg colour		Beak colour	
		Black	Brown	White	Black	Yellow	Black	Yellow	Black	Yellow
Phieng Cam	446	14.78	70.66	14.56	4.5	95.5	94.65	5.35	96.57	3.43
Chieng Noi	142	18.31	65.49	16.20	9.86	90.14	95.77	4.23	96.48	3.52
Chieng Chan	185	15.68	71.89	12.43	3.78	96.22	97.84	2.16	97.84	2.16
Overall	773	15.62	70.03	14.36	5.29	94.71	95.59	4.41	96.85	3.15

Genetic variation between populations: Genetic relationships among populations and feather colour variants were determined by multi locus estimator of F_{ST} (Proportion of genetic variability due to population differences) between all pairs of samples using Weir and Cockerham estimations. Pairwise tests of differentiation between populations were done using the Bonferroni procedure of FSTAT software with 3000 permutations.

Pairwise genetic distances were calculated using Nei's standard genetic distance (Nei, 1972), Cavalli – Sforza's chord measure (Cavalli - Sforza and Edwards, 1967) and Reynolds' genetic distance (Reynolds *et al.,* 1983) using PHYLIP computer package version 3.5 (Felsenstein, 1993). Based on Nei's standard genetic distance, a phylogenetic tree was constructed using the Neighbor – Joining method of PHYLIP computer package version 3.5. Bootstrapping was performed with 1000 re-samplings to test the robustness of the tree.

A model based algorithm implemented in software package STRUCTURE was used to cluster individuals from multi locus genotypes (Pritchard *et al.,* 2000). Analysis involved an admixture model with correlated allele frequencies. The model was tested using a 20 000 iterations burn-in phase and 50 000 iterations for 2 = K = 4 assumed clusters with 100 runs for each *K* value. A pairwise comparison of the solutions was done using SIMCOEFF with solutions over 95% similarity considered as identical. The most frequent of these pair wise comparisons were considered to be the most probable clustering and were visualized using DISTRUCT programme (Rosenberg, 2004).

Results

Chicken management practice: In Chieng Chan all households provided chicken houses, whereas 90% and 66 % of the households in Chieng Noi and Phieng Cam provided housing, respectively. Housing structures were made from wooden or bamboo poles and thatched roofs and had hanging nests for laying hens. The non-housed chickens mostly perched around farmer's houses or on trees near the farmer's homesteads and layed eggs in nests placed behind farmer's homestead. No households had records on chicken survival and mortality rates. In addition, they did not vaccinate their chickens nor use veterinary drugs for treatment or prophylaxis. The chickens in all households scavenged for a variety of feedstuffs that included cereals, weeds, seeds, insects, worms and various herbs. Farmers in the three villages supplemented their chickens with whole maize mostly two times a day, in the morning and then in the afternoon. The amount of feed supplement given was not recorded but varied for the whole flock at any feeding time and was indiscriminate of age group. Other farm animals such as pigs competed for the same supplement. All the households interviewed declared that women owned chicken. Chicken meat was used for consumption by household members and special guests during ceremonial gatherings, such as the marriage feast and funerals. The meat was also an important meal for women during the first month after childbirth. All eggs were kept for incubation by brooding. Farmers did not sell chicken products to generate cash income.

Flock characteristics and phenotypic characterization: A total of 773 chickens that comprised of 25.31% chicks, 38.66% growers and 36.02% mature cocks and hens were observed in the study. Flock size per household averaged 14.44 ± 7.38 birds. The three feather colour variants were found in all households. The majority (87.7%) of chickens were hatched from within the household flocks, while 7.78 % were gifts from neighbours and 5.56 % brought in as gifts from relatives, respectively. Just as the chickens hatched at the household, those brought in as gifts were also included in the farmer's breeding stock. None of the households exchanged breeding cocks.

Two types of comb shapes were observed in the 3 village based populations. Single comb dominated with a frequency of 96.42 % while 3.58% of the chickens had rose comb shape. The frequencies of feather, skin, leg and beak colours are shown in Table 1. The body weight of mature birds averaged 1617g (SE = 52). The estimated age at start of laying was 28 weeks and mean clutch size was 12 eggs. Hatchability ranged from 81.69 to 84.72% in the three villages.

Microsatellite markers allele distribution: The observed characteristics of the 29 microsatellite loci are shown in Table 2. A total of 186 alleles were observed across all populations. The average number of alleles per locus was 6.42 ± 3.25 and ranged from 2 (MCW103 and MCW222) to 15 (LEI234). Within a marker, allele size range varied from a difference of 2 base pairs (bp) (220 – 222bp) for locus MCW222 to 144 bp (220 – 364bp) for locus LEI 234. In 21 of the 29 loci, a total of 13, 16 and 15 private alleles were found in Phieng Cam

Table 2: Characteristics of microsatellite loci: reported and observed number of alleles and ranges of base pairs

Locus	Reported* allele range (bp)	Reported No. of alleles	Observed No. of alleles	Observed allele size range (bp)
ADL 0112	120-134	8	5	124[3]126 128[1r] 130 132[2l]
ADL 0268	102-116	8	6	104 108 110 112[3] 114 116
MCW 0330	256-300	11	5	258 270 278 288 290
MCW 0295	88-106	10	8	90[3] 92 98 100 104 106 108[r] 110[3r]
MCW 0248	205-225	11	5	215 217 219 221[3] 223
MCW 0222	220-226	4	2	220 222
MCW 0216	139-149	6	4	141 143 145 147[2l]
MCW 0206	221-249	15	7	221[2] 223 227[2l] 229 231 233 243
MCW 0183	296-326	14	8	296 298 302 304 306[1r] 312 318 320[w]
MCW 0165	114-118	3	3	114 116 118
MCW 0123	76-100	12	9	76 82 84 86[1] 88 90 92 94 98[3l]
MCW 0111	96-120	13	7	100 102 104 106 108[2] 110 114
MCW 0104	190-234	10	11	190 194[3r] 196[2r] 200 202 206 210[3l] 222[2]224 226 230[2w]
MCW 0103	266-270	2	2	266 270
MCW 0098	261-265	3	3	263 265 267[1w]
MCW 0081	112-135	10	5	114 116 121 135 143[2w]
MCW 0080	266-282	8	8	266 268 270[1l] 272 276[3w] 278 280 282
MCW 0078	135-147	5	3	139 141 143
MCW 0069	158-176	10	9	158 162 164 166 168[1] 170 172 174[2r] 176[w]
MCW 0067	176-186	6	4	178 180 182 184
MCW 0037	154-160	4	3	154 156 158
MCW 0034	212-246	18	11	214[1r] 220 222[w] 224[2w] 226 228 230 232 238 240 242[1l]
MCW 0020	179-185	4	5	179 181 183 185 187[1]
MCW 0016	162-206	15	7	170 172 174 178 180 182[1r] 184[3w]
MCW 0014	164-182	23	8	158[3r] 164 168 170[1w] 174 176 178 186[3l]
LEI 0234	216-364	23	15	220 268[3l] 276 284[3l] 288 292 296 300 304 308 312 344[2] 356[1l] 360[2r] 364[r]
LEI 0166	354-370	8	5	356 360 362[l] 364 366
LEI 0094	247-287	21	13	247[1l] 249 255[3] 259[2] 261 263[w] 265 267[2] 269[2l] 273[w] 279 281[2r] 283
ADL 0278	114-126	7	5	114 118 120 122 124*

Comparisons were made with the AVIANDIV populations

[1,2,3]Private alleles for Phieng Cam population, Chieng Noi population and Chieng Chan populations.

[l,r,w]Private alleles for black, brown including multicolour, white feather colours

population, Chieng Noi population and Chieng Chan population, respectively. Fourteen, 13 and 11 alleles were specific for black, brown and white feather colour variants, respectively.

Genetic diversity and Hardy–Weinberg testing: The observed and expected heterozygosities, F_{IS} and the Hardy–Weinberg equilibrium test for the 29 loci across all populations are presented in Tables 3 and 4. Two microsatellite markers (MCW123 and LEI234) deviated ($P<0.05$) from Hardy-Weinberg equilibrium (Table 3). Overall allele frequencies of three village based populations did not deviate ($P>0.05$) from Hardy-Weinberg equilibrium (Table 4).The average inbreeding coefficient (F_{IS}) was 0.044, and the overall F_{IT} and F_{ST} was 0.069 and 0.026, respectively.

Population differentiation and relationship: Pairwise F_{ST} values among villages are shown in Table 5. Small population divergence was observed among both the village and plumage colour based populations. The lowest F_{ST} value was between Phieng Cam population and Chieng Noi populations. Significant differences ($P<0.05$) were observed between Chieng Chan and Phieng Cam and Chieng Noi populations. There was no significant difference ($P>0.05$) between the three colour variants.

Genetic distance and phylogenetic tree: The Cavalli-Sforza's chord, Reynolds' and Nei's genetic distances among the village based populations are shown in Table 6. The closest genetic distance was between Phieng Cam and Chieng Noi populations for all the three estimates. The Neighbour joining tree reconstructed from Nei's genetic distance is shown in Fig. 1.

The clustering of H'mong chickens is illustrated in Fig. 2. The most number of identical solutions were observed at K = 2 in which 10 out of 100 runs were identical at 95% threshold. At K = 3 only 2 runs were identical at 95 % while a reduction of threshold to 90% yielded 5 identical runs. K = 4 failed to yield identical runs even at 75 % threshold and only 4 runs were considered 70 % similar to each other.

Discussion

The observation that all chickens scavenged for a variety of feed is similar to results reported by Maphosa *et al.* (2004) and Muchadeyi *et al.* (2004). This method of feed supplementation does not make the best use of limited

Table 3: Observed (Ho) and expected (He) heterozygosity, F_{IS}, F_{IT}, F_{ST} and Hardy-Weinberg-Equilibrium (HWE) test for each marker per population

Locus	Ho	He	F_{IS}	F_{IT}	F_{ST}	Test HWE
ADL0112	0.444	0.508	0.124	0.118	-0.007	NS
ADL0268	0.750	0.768	0.023	-0.006	-0.030	NS
MCW0330	0.667	0.662	-0.008	0.046	0.053	NS
MCW0295	0.639	0.660	0.033	0.026	-0.006	NS
MCW0248	0.639	0.705	0.093	0.098	0.005	NS
MCW0222	0.258	0.460	0.444	0.455	0.019	NS
MCW0216	0.361	0.379	0.047	0.028	-0.020	NS
MCW0206	0.806	0.668	-0.206	-0.144	0.052	NS
MCW0183	0.611	0.562	-0.088	-0.066	0.020	NS
MCW0165	0.528	0.630	-0.066	0.169	0.008	NS
MCW0123	0.667	0.828	0.195	0.202	0.009	*
MCW0111	0.722	0.672	-0.075	0.050	0.117	NS
MCW0104	0.778	0.749	-0.039	-0.032	0.006	NS
MCW0103	0.528	0.500	-0.056	-0.065	-0.009	NS
MCW0098	0.472	0.378	-0.251	-0.212	0.031	NS
MCW0081	0.444	0.472	0.059	0.025	-0.036	NS
MCW0080	0.556	0.628	0.115	0.288	0.196	NS
MCW0078	0.528	0.564	0.065	0.110	0.049	NS
MCW0069	0.806	0.790	-0.019	0.039	0.057	NS
MCW0067	0.667	0.678	0.017	0.049	0.033	NS
MCW0037	0.500	0.640	0.219	0.274	0.070	NS
MCW0034	0.722	0.736	0.019	0.032	0.013	NS
MCW0020	0.722	0.721	-0.002	0.016	0.017	NS
MCW0016	0.639	0.668	0.043	0.061	0.019	NS
MCW0014	0.750	0.802	0.061	0.033	-0.034	NS
LEI0234	0.750	0.896	0.163	0.174	0.013	*
LEI0166	0.528	0.643	0.179	0.206	0.033	NS
LEI0094	0.972	0.845	-0.151	-0.126	0.022	NS
ADL0278	0.556	0.630	0.118	0.095	-0.026	NS
Mean	0.621	0.650	0.044	0.069	0.026	

NS: Not significant value (P> 0.05), * Significant value (P= 0.05)

resources. Younger and weaker birds have to compete with mature birds and other scavenging farm animals such as pigs for the scarcely available supplements. In addition to inadequate nutrition, the husbandry practices expose chickens to diseases. However, data on mortality and morbidity of chickens could not be recorded within the given frame work of this study.

The ownership of H'mong chickens by women, suggests a gender bias in chicken production and corresponds with the findings of Kitalyi (1998), who recognized the importance of village chicken for women. McAinsh *et al.* (2004) established that unlike large species that are owned by men, chickens are directly accessible to women. There could be several reasons to explain why chickens in most farming systems are owned by women. Firstly, chickens are considered a farm asset of less economic value and, as such, men sideline it in favor of larger species. Women on the other hand have been shown to use chickens for household food security (Gueye, 2002). In India, newly married women would maintain ties with their original and new families through keeping their chickens in these households' flocks (Kumtakar, 2000). Based on these and results from other studies it would be most appropriate for this gender bias to be considered when designing either developmental or conservation projects for village chickens.

The greater importance of H'mong chickens as a food supplier for one's own household in comparison to the generation of income agrees with the findings of Muchadeyi *et al.* (2005). Although farmers invested little in chicken production, they are in most cases able to derive meat from H'mong chicken production to meet their food needs. It remains to be investigated to what extent the contribution of local chickens to the human diet could be improved through intensified production. The observed average flock size in this study (14.44± 7.38) was lower than reported from other countries with comparable production systems. For example, 26 birds was reported in Pakistan (Javet *et al.*, 2003) while 22 birds per flock were observed in Senegal (Missohou *et al.*, 2002). The mean flock sizes was, however, higher that of comparable to that of 12.9 birds recorded in local Malawi chickens (Gondwe and Wollny, 2004). Such differences in flock sizes probably reflect the subsistence role of H'mong chickens. Chickens were reared for both home consumption and commercial purposes in the other mentioned countries.

The observed estimated age at the start of laying for H'mong chicken agrees with Nguyen Van Tru (2000)

Table 4: Mean number of alleles/locus, observed (Ho) and expected (He) heterozygosity, F_{IS} and Hardy-Weinberg-Equilibrium (HWE) test per chicken population

Population	Mean alleles/locus	Ho	He	F_{IS}	HWE
Phieng Cam	4.79 (1.95)	0.603 (0.03)	0.650 (0.03)	0.074	NS
Chieng Noi	5.10 (2.48)	0.668 (0.02)	0.668 (0.03)	0.001	NS
Chieng Chan	4.86 (1.94)	0.592 (0.03)	0.627 (0.03)	0.059	NS

The number in parenthesis is the standard deviation. NS: Not significant

Table 5: Pairwise F_{ST} comparison between the three chicken populations, obtained after 3000 permutations

Population	Chieng Noi	Chieng Chan
Phieng Cam	0.0105	0.0343*
Chieng Noi		0.0334**

* significance at the 5% level, ** significance at the 1% level.

who reported an age at the start of laying of 197 days (28 weeks) for 'Meo' scavenging chickens in Cao Bang Province. However, Vo Van Su *et al.* (2001) reported an age at the start of laying of H'mong chickens of 144 days (16 weeks). The differences are probably due to variation in the type of management systems used in the experiments with some observing under scavenging system and others intensive (on station) systems. Variations might also be due to differences in the genetics of the birds as was observed by Aganga *et al.*, (2000) and Benabdeljelil and Arfaoui (2001) in Botswana and Morocco, respectively.

The number of eggs per clutch found in this study (12 eggs on average) was close to those reported in other reports (Nguyen Van Tru, 2000; Benabdeljelil and Arfaoui, 2001). The higher hatchability observed in H'mong (82% to 85%) chickens compared to other reports for village chickens might be due to the differences in the climate experienced in the study areas (Aganga *et al.*, 2000; Benabdeljelil and Arfaoui, 2001; Maphosa *et al.*, 2004). The high humidity and relatively lower temperature in the cold mountains might have positively affected hatchability of H'mong chickens.

Regarding the assessment of genetic diversity using molecular markers, the mean number of alleles observed in this study compare to those reported by Marle-Köster and Nel (2000) for South African chickens. However, Romanov and Weigend (2001) reported a higher mean number of alleles for domestic and jungle fowl populations in Ukraine and Germany. The heterozygosity estimate is higher than the reported estimates of Marle-Köster and Nel (2000), Wimmers *et al.* (2000), Vanhala *et al.* (1998) and Zhou and Lamont (1999). H'mong chicken population tested in this study showed a relatively high genetic variability compared to other findings. The low inbreeding in H'mong chicken populations might be explained by two factors. Firstly, H'mong chicken flocks freely roam during the day when they scavenge for feed. In addition, H'mong households are structured according to their clans, in which related families live in close proximity. Households within a clan reared their chickens as one big flock. Random mating with chickens of different flocks would increase effective population size and minimize inbreeding.

Genetic differences between breeds and populations are controlled by mutation, genetic drift, selection and migration (Eding and Laval, 1999). The overall genetic differentiation observed in this study is low indicating little genetic effects of drift or mutation in the sub-populations. About 2.6 percent of the total genetic variation was due to between population variation which is small compared to the 97.4 % found within the sub populations themselves. These findings imply high levels of gene flow among villages resulting in admixed populations. Interbreeding of chickens from different villages can explain the non-significant F_{ST} between chickens evaluated from neighbouring villages Phieng Cam and Chieng Noi compared to the chickens from distant Chieng Chan village. The implied gene flow is not, however, supported by survey data that showed low levels of chicken exchanges between households. The insignificant F_{ST} values of the feather colour variants are expected because farmers keep chickens of different plumage colour within the same flocks resulting in possible inter-mating.

The three measures of genetic distance showed different distances because they are based on different scaling systems. All assume genetic drift in causing population divergence. For small and sub-structured populations such as the H'mong chickens that have possibly not been separated for a long time, distances that factor drift and not mutation become more appropriate. Regardless of the differences in numerical values all three distances gave similar trends that correlate with the geographical distances among the populations.

One major weakness of genetic distances and resulting phylogenies is the use of predefined populations to determine population structure. In contrast, the STRUCTURE software uses multi locus genotypes to infer the structure of individual chickens. The highest number of identical solutions were obtained at K = 2 which agree with the F_{ST} and other genetic distance measures. However the number of identical solutions out of the 100 runs were quite low implying weak demarcations between the three village based populations. Even at K = 2 majority of the Chieng Chan chickens were assigned to the cluster with Phieng Cam and Chien Noi chickens (Fig. 2). Such a weak sub-structuring is expected considering the relatively low

Table 6: Genetic distance matrices between pairs of chicken populations

Population	Chieng Chan			Chieng Noi		
	Nei[1]	Cavalli–Sforza[2]	Reynolds[3]	Nei	Cavalli–Sforza	Reynolds
Phieng Cam	0.1519	0.0932	0.0820	0.1013	0.0747	0.0534
Chieng Noi	0.1436	0.0810	0.0762			

[1] Nei's standard genetic distance. [2] Cavalli – Sforza's chord measure. [3]Reynolds' genetic distance

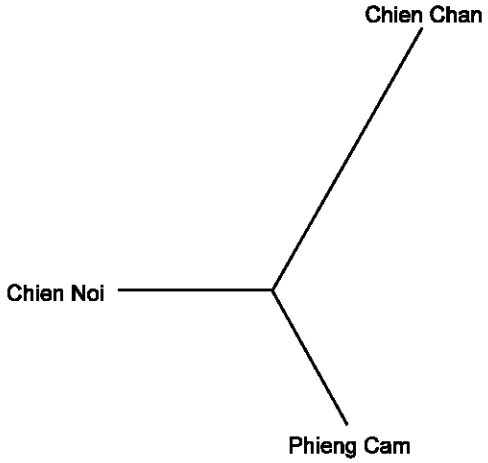

Fig. 1: Phylogenetic tree of chicken populations of three villages, reconstructed based on Nei's standard genetic distance and Neighbour-Joining method

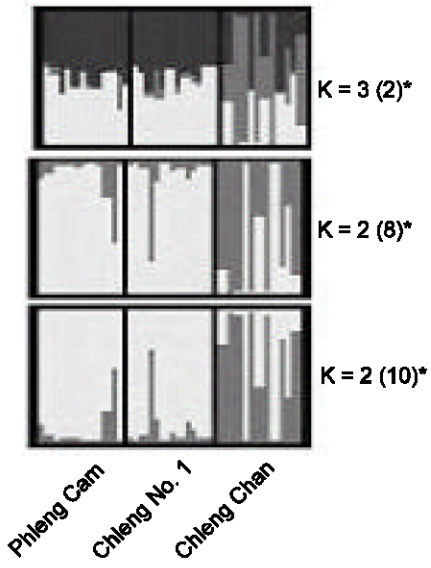

Fig. 2: Clustering of H'mong chicken populations using STRUCTURE software

*the number in parenthesis indicates the number of runs having a similarity coefficient ≥ 95%

geographic distances among villages that could allow exchange of chickens. Gene flow between scavenging populations usually happens without the knowledge of the farmer. This explains why the results of both STRUCTURE and pairwise F_{ST} contrasts with information given by households during interviews. Molecular data provides more accurate information on population structures and breeding patterns of village chickens compared to surveys that depend on farmers' observations and recall.

Like most indigenous livestock resources H'mong chickens are raised extensively under compromised management, a practice that could impact negatively on their existence. Lack of significant genetic difference among the three plumage colour variants suggests that plumage colour cannot be effectively used to select populations for conservation of genetic diversity. Diversity of H'mong chickens tend to be influenced by geographical locations.

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References

Aganga, A.A., U.J. Omphile, P. Malope, C.H. Chabanga and L.G. Motsamai, 2000. Traditional poultry production and commercial broiler alternatives for smallholder farmers in Botswana Livestock Research for Rural Development, 12. http://www.cipav.org.co/lrrd/lrrd12 /4/Aga124a.htm.

AVIANDIV; Weigend, S (Coordinator), M.A.M. Groenen, M. Tixier-Boichard, A. Vignal, J. Hillel, K., Wimmers, T. Burke and A. Mäki-Tanila, 1998-2000. Development of Strategy and Application of Molecular Tools to Assess Biodiversity in Chicken Genetic Resource. EC Contract No. BIO4-CT98-0342 (1998-2000); http://w3.tzv. fal. de/ aviandiv.

Benabdeljelil, K. and T. Arfaoui, 2001. Characterization of Beldi chicken and turkeys in rural poultry flocks of Morocco. Current state and future outlook. Animal Genetic Resources Information, 31: 87-95.

Crooijmans, R.P.M.A., P.A.M. Vanoers, J.A. Strijk, J.J.V.D. Poel and M.A.M. Groenen, 1996. Preliminary linkage map of the chicken (*Gallus domesticus*) genome based on microsatellite markers: 77 new markers mapped. Poult. Sci., 75: 746-754.

Cavalli-Sforza, L.L. and K.J. Edwards, 1967. Phylogenetic analysis. Models and estimation procedures, Am. J. Human Genet., 19: 233-257.

Eding H. and G. Laval, 1999. Measuring the genetic uniqueness in Livestock. In Genebanks and the conservation of farm animal genetic resources. J. K. Oldenbroek (editor). DLO Institute of Animal Science and Health. The Netherlands, pp: 33-58.

FAO., 1995. Global project for the maintenance of domestic animal genetic diversity (MoDAD). Draft project formulation report. Food and Agriculture Organization of United Nations, Rome.

Felsenstein, L., 1993. Phylip (Phylogeny Inference Package) Version 3.5c. University of Washington, Washington, D.C.

Goudet, J., 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). http://www.unil.ch/izea/softwares/fstat.html.

Gondwe T.N. and C.B.A. Wollny, 2004. With Globalisation will low-input production systems prevail in Malawi? The case of rural chicken. Book of Abstracts of the 55th Annual meeting of the European Association for animal Production. September 5th-9th 2004. Bled, Slovenia. Wageningen Academic publishers. The Netherlands, pp: 338.

Gueye, F.H.E., 2002. Employment and income generation through family poultry in low income food-deficit countries. World's Poult. Sci. J., 58: 541-555.

Hoang Van Tieu and Vo Van Su, 2000. Vietnamese domestic animal genetic resources utilization–some aspects. The closing conference for genetic resources conservation on plant, animal and microbial 1996–2000 and oriented objectives for the period of 2001-2005 in Vietnam. December 2000, Hanoi, Vietnam, 297-203.

Hoang Van Tieu, Le Viet Ly, Vo Van Su and Le Minh Sat. 2001. Summary on Vietnam Livestock Genetic Resources Conservation (http://www.vcn. vnn.vn/qg/), National Institute of Animal Husbandry, Vietnam.

Javet, K., M. Farooq, M.A. Mian, F.R. Durrani and S. Mussawar, 2003. Flock size and egg production performance of backyard chicken reared by rural woman in Peshawar, Pakistan. Livestock Research for Rural Development, 15. http://www.cipav.org. co/lrrd/ lrrd15/ 11/ jave 1511.htm.

Kitalyi, A.J., 1998. Village chicken production system in rural Africa. Household security and gender issues. Animal Production and Health Paper 142. Food and Agriculture Organization of United Nations, Rome.

Kumtakar, V.A., 2000. Backyard poultry system in the tribal areas of Shanpura Block in Jabalpur District, Madhya, Predesh, India. In: Poultry as a Tool in Poverty Eradication and Promotion of Gender Equity (F Dolberg and P. H. Peterson eds). Proceedings of an International Workshop. March 22-26, 1999. Tune. Landboskole. Denmark.

Marle-Köster, E.V.M. and E.L.H. Nel, 2000. Genetic characterization of native southern African chicken population: Evaluation and selection of polymorphic microsatellite markers. S. Afr. J. Anim. Sci., 30: 1-6.

Maphosa, T., J. Kusina, N.T. Kusina, S. Makuz and S. Sibanda, 2004. A monitoring study comparing production of village chickens between communal (Nharira) and small-scale commercial (Lancashire) farming areas in Zimbabwe. Livestock Research for Rural Development 16, http://www.cipav. org.co/lrrd/ lrrd16/maph 16048. htm.

McAinsh, C.V., J. Kusina, J. Madsen and O. Nyoni, 2004. Traditional chicken production in Zimbabwe. World'-s Poult. Sci. J., 60: 233-246.

Missohou, M., P.N. Dieye and E. Talaki, 2002. Rural poultry production and productivityin southern Senegal. Livestock Research for Rural Development 14, www.cipav.org.co/ lrrd / lrrd 14/ 2 /miss142.htm.

Muchadeyi, F.C., S. Sibanda, N.T. Kusina, J. Kusina and S. Makuza, 2004. The village chicken production system in Rushinga District of Zimbabwe. Livestock Research for Rural Development 16. www.cipav.org.co/ lrrd/ lrrd16/ 6/cont1606.htm.

Muchadeyi, F.C., S. Sibanda, N.T. Kusina, J. Kusina and S.M. Makuza, 2005. Village chicken Flock dynamics and the contribution of chickens to household livelihoods in a smallholder farming area in Zimbabwe. Tropical animal health and production, 37: 333-344.

Nei, M., 1972. Genetic distance between populations. American Naturalist ,106, 283-292.

Nguyen Van Tru, 2000. Biological charaterization and productivity of "Meo" chicken in Cao Bang Province of Vietnam. Msc thesis. Forestry and Agriculture University of Thai Nguyen, Vietnam.

Phuong T. and M. Hoang, 2003. H'Mong chicken – Precious genetic resource conserved. Economic and Life Newpapers 352, Hanoi, Vietnam.

Pritchard J. K., M. Stephens and P. Donnelly, 2000. Inference of Population Structure Using Multilocus Genotype Data. Genetics, 155: 945-959.

Raymond, M. and F. Rousset, 2004. Genepop on the web. http://wbiomed.curtin.edu.au/genepop/.

Reynolds, J., B.S. Weir and C.C. Cockerham, 1983. Estimation of the co-ancestry coefficient basis for a short-term genetic-distance, Genetics, 105: 767-779.

Romanov, M.N. and S. Weigend, 2001. Analysis of genetic relationships between various populations of domestic and Jungle fowl using microsatellite markers. Poult. Sci., 80: 1057-1063.

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

Statistical Analysis Systems (SAS), 1999. SAS/STAT user's guide, version 8.5. SAS Institute Inc. Cary, North Carolina.

Sonla Statistic Department, 2005. Sonla Statistic Yearbook. April 2005, pp 5 - 8.

Vanhala, T., M. Tuiskula-Haavisto, K. Elo, J. Vilkki and A. Mäki-Tanila, 1998. Evaluation of genetic variability and genetic distance between eight chicken lines using microsatellite markers. Poult. Sci., 77: 783-790.

Vo Van Su, Ngo Thi Kim Cuc, Le thi Binh, Vu Khanh Van, Pham Cong Thieu, Luong Thi Hong, 2001. Adaptation of H'mong chicken in ex-situ conservation. Ending report for project on conservation of Vietnamese livestock breeds having outstanding economic traits. National Insitute for Animal husbandry, Hanoi, Vietnam.

Wimmers, K., S. Ponsuksili, T. Hardge, A.Z. Valle, P.K. Mathur and P. Horst, 2000. Genetic distinctness of African, Asian and South American local chickens. Anim. Genet., 31: 159-165.

Zhou, H. and S.J. Lamont, 1999. Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers. Animal Genetics, 30: 256-264.