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Genetic variability and phylogenetic relationship establishes distinctness of Kaunayen chicken of Manipur

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ABSTRACT

Conservation of locally adapted indigenous livestock has become an important objective in sustainable animal breeding.The current study is first detailed analysis of the genetic diversity harboured by Kaunayen chicken of Manipur.The genotype data generated on 24 microsatellite markers was analysed to establish distinctness of Kaunayen from other registered poultry breeds of India. Large number of observed alleles (212) and heterozygosity (0.66) indicated high genetic diversity. Mean number of alleles observed in Kaunayen chicken were 8.83±0.31 and ranged between 4 (MCW250 and LEI174) and 15 (LEI120). Mean effective number of alleles was significantly less (4.11±0.38) than the observed number of alleles. The maximum observed heterozygosity (1.0) was observed in MCW262 locus and the minimum (0.368) in LEI166. The expected heterozygosity (0.706) was more than the observed heterozygosity (0.664±0.036) which points to heterozygote deficiency and was also reflected in positive F_{IS} estimate (0.06) for the population. Non-significant heterozygote excess on the basis of Infinite allele model and Two-phase model in conjunction with mode-shift analysis test, indicated an absence of bottleneck. Phylogenetic reconstruction on the basis of genetic distance places Kaunayen chicken as a distinct population with respect to other poultry breeds of India. All analysis showed that a significant amount of genetic variation is maintained in Kaunayen chicken population and has appropriately been registered as the 17th chicken breed of India.

Key words: Bottleneck, Diversity, Heterozygosity, Kaunayen chicken, Phylogenetic studies, SSR markers

The world is becoming aware of the shortcomings regarding the adaptability and evolutionary potential of highly industrialized livestock breeds, considering global climate change and food security. Native livestock breeds generally possess adaptive characteristics that make them better suited to local environmental (often extreme) conditions (Sharma *et al.* 2015). These breeds represent a unique genetic resource for long-term and sustainable animal genetic improvement (Medugorac *et al.* 2009). However, many indigenous breeds remain poorly characterized and are currently threatened by extinction due to changing production systems, preferring exotic commercial breeds and indiscriminate crossbreeding. To prevent the irreversible erosion of animal genetic resources that might compromise future breeding programmes, the FAO initiated the Global Plan of Action for Animal Genetic Resources to facilitate the characterization and conservation of indigenous livestock breeds (FAO 2007a).Chicken genetic resources are probably the most endangered and under-conserved of all livestock species, with approximately 33% of the world's chicken breeds considered endangered (FAO 2007b).

In India, indigenous chickens are raised by smallholder farmers with little resources and are considered important genetic resources that should be conserved against production threats and replacement with commercial hybrids. Characterization of these genetic resources will serve as an essential prerequisite for the identification and effective management and utilization of indigenous chickens, which will facilitate their conservation. The adaptive features, traits of scientific and economic interest, cultural-historical values, strong links to regional traditions and ability to generate income associated with most of these village chicken populations further justify conservation efforts. For this reason, phenotypic observations or monitoring of productive traits combined with molecular analysis can be useful information for conservation decisions (Mtileni *et al.* 2016).

One such population is Kaunayen, found in Manipur, a north eastern state of India. It spreads over one among the two global biodiversity hotspots (Eastern Himalaya comprising of states of east/north-east India and Western ghat) of the country. The word 'Kaunayen' is a combination of two words - 'Kauna' and 'yen'. In Manipuri language,

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Kauna means 'kick/fighting' and yen means 'hen/poultry'. These birds are prized for their 'martial qualities' and not for meat. Kaunayen birds have elongated body with long neck and long legs. The predominant plumage colour is black followed by brown (or red) with or without patches of white, black, brown or golden feathers on neck, back and wings especially in males. Few grey, white or golden yellow birds are also observed. Hens are generally black, grey, blackish grey or whitish grey with few brown feathers on neck, breast and wings (Vij *et al.* 2016).This bird contributes a lot in generating income for the poultry keepers due to its fighting qualities. Apart from this, it also provides nutritional security through eggs and meat, thereby contributing immensely to the economy of the people of this area. However, no information is available on the diversity characteristics of Kaunayen birds.

Several studies have been conducted to assess chicken genetic diversity using microsatellite markers and the reported results are clear evidences of the usefulness of these panels for biodiversity studies (Kaya and Yilidiz 2008, Wilkinson *et al.* 2012, Suh *et al.* 2014). Also, the microsatellite loci are considered the best markers for detecting recent bottlenecks (Cornuet and Luikart 1996). Therefore, the goal of present study was to investigate the genetic variation in the Kaunayen birds using microsatellite markers and to utilize the generated data to carry out genetic bottleneck analysis as well to establish the phylogenetic relationship with registered breeds of Indian poultry.

MATERIALS AND METHODS

Blood sample collection and DNA extraction: Blood samples were collected from the brachial vein of the adult birds. A total of 38 Kaunayen chicken from whole of the Imphal Valley comprising of Thoubal, Imphal West, Imphal East and Bishnupur districts of Manipur were sampled. Within each district, samples were collected from several birds from multiple households in different villages. To minimize the chances of relatedness among the birds selected from one village, a single bird was used from each household. The households within each village from which each bird was used were approximately 0.5 to one mile apart.

Genomic DNA was extracted from 2 ml of blood using QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany). DNA integrity and quantity was checked on 1% agarose gel by direct comparison with a standard marker (Low DNA Mass Ladder - Gibco.Brl), and in a spectrophoto meter (Nano Drop ND-1000 Spectrophotometer - Thermo Fisher Scientific).

Microsatellite genotyping: A panel consisting of 24 microsatellite markers was selected for the diversity analysis of Kaunayen population. These were chosen from literature aiming to analyze highly polymorphic markers spread across the genome. These primers have previously been used for indigenous chicken diversity studies (Tantia *et al.* 2006) and hence the data can be compared with that of 16 Indian poultry breeds for estimating the phylogenetic relationship. These markers also adhere to the guidelines of International Society for Animal Genetics and FAO (http: //dad.fao.org/en/refer/library/guidelin/marker.pdf). Detailed information on primers is presented in Table 1. Forward primer of each marker was 5' labeled with either FAM or HEX fluorescent dye. PCR amplification was performed in a reaction volume of 15 µl on i-cycler. Reaction mixture consisted of 50 ng of genomic DNA, 200 µM of each dNTP, 50 pM of each primer and 0.75 units of *Taq* DNA polymerase. A negative control, consisting of all the reaction components, except for the template DNA, was also included to monitor any possible contamination. The amplification protocol consisted of initial denaturation of 94°C for five minutes; 32 cycles of 95°C for 30 sec, specific annealing temperature for 45 sec, 72°C for 45 sec and final extension step at 72°C for 10 min. The amplification products were electrophoresed on a 1.8% agarose gel treated with ethidium bromide (0.5 mg/ml) for visualization of DNA bands under ultraviolet light. PCR products were multiplexed and genotyping was carried out on an automated ABI-3100 DNA sequencer (Applied Biosystems, USA) using LIZ 500 as the internal size standard (Applied Biosystems, USA). Allele sizing was done using GeneMapper^{TM} software v 3.7. Stutter related scoring error, often seen in dinucleotide repeats, was absent and alleles could be scored unambiguously.

Statistical analysis of genotype data: Basic genetic parameters including allele frequencies, observed (Na) and effective number of alleles (Ne), observed (Ho) and expected heterozygosity (He) and heterozygote deficit (F_{IS}) in the whole population were calculated by analyzing the genetic data with GenAlEx 6.2 software (Peakall and Smouse 2008). Tests of Hardy-Weinberg equilibrium and Ewens-Watterson Neutrality were applied using POPGENE 1.31 version (Yeh *et al.* 1999). The PHYLIP 3.6 (Felsenstein 1993) was utilized to estimate the genetic distances on the basis of genotype data generated by utilizing the same set of 24 microsatellite markers among the Kaunayen and other Indian poultry breeds/ populations. Nei's genetic distance and Cavalli-Sforza Chord Measure were estimated. Pair wise matrix of the genetic distances was then used to obtain Neighbor joining (NJ) tree which was visualized using the software TreeView (Page 1996). Bottleneck events in the population were tested by three methods. The first method consisted of three excess heterozygosity tests developed by Cornuet and Luikart (1996), viz. Sign test, Standardized differences test, and a Wilcoxon sign-rank test. The probability distribution was established using 1,000 simulations under three models–Infinite allele model (IAM), stepwise mutation model (SMM) and two-phase model of mutation (TPM). The second method was the graphical representation of the mode-shift indicator originally proposed by Luikart *et al.*1998. Loss of rare alleles in bottlenecked populations is detected when one or more of the common allele classes have a higher number of alleles than the rare allele class (Luikart *et al.* 1998). These two methods were applied using Bottleneck v1.2.02 (http:// www.ensam.inra.fr/URLB).

RESULTS AND DISCUSSION

To formulate appropriate breeding strategies for indigenous chicken population of Manipur (Kaunayen) and to prevent any genetic erosion, it is necessary to assess the present level of genetic diversity in the population.

Microsatellite polymorphism and allelic variability: An exact test for genotypic linkage disequilibrium yielded no significant *P* values across the population, and therefore independent assortment of all the loci was assumed. Reasonable polymorphism in Kaunayen poultry was evident from the allele frequency data (Available on request). All the markers were polymorphic and a total of 212 alleles were detected across the 24 loci. LEI120 showed the highest number of observed alleles per locus (15) while MCW250 and LEI174 showed the lowest (4) with the mean number of alleles (MNA) of 8.833 (Table 1).

Expected number of alleles varied from 1.656 (LEI122) to 9.857 (LEI155) with the mean of 4.11. The use of microsatellites with a range of polymorphism reduced the risk of overestimating genetic variability, which might occur with microsatellite exhibiting only high polymorphism. According to standard selection of microsatellites loci (Barker 1994), it has been suggested that microsatellite preferably should have at least 4 alleles to be useful for the evaluation of genetic diversity, therefore all the 24 microsatellites were retained for further analysis. Moreover, the selected markers were present on different chromosomes thus were unlinked and represented a large region of chicken genome. No microsatellite from W or Z chromosome was included as majority of mapped microsatellites have been reported to be incorrectly assigned (Ben-Avraham *et al.* 2006). Shannon's information Index (I) is a parameter indicative of the informative degree of a marker and is shown for all the markers in Table 1. I value in this study ranged from 0.8445 (LEI166) to 2.405 (LEI155). Most of the markers had high I values and thus can potentially be used for diverse genetic applications including linkage mapping, individual identification and parentage testing.

The results suggest existence of enough genetic variation in the Kaunayen population for further breeding programs. The number of alleles and allele size range observed in the present investigation (Table 1) were broadly in agreement with the literature on indigenous poultry. Vijh and Tantia (2004) reported an average of 8.7 alleles in indigenous poultry based on 26 microsatellite loci ubiquitously distributed throughout the genome. It is evident from the mean number of alleles (8.83) that Kaunayen has comparable genetic variation as of recognized breeds of India. In another study, much higher genetic variation was reported in the registered breeds of chicken in India (15.88, Tantia *et al.* 2006). Much higher observed number of alleles ranging from five (MCW0111) to forty-three (LEI0212) with an average number of 19 alleles per locus was reported in Indian red jungle fowl from northern India and three

Table 1. Allele size range, observed (Na) and effective number of alleles (Ne), Shannon's Information index (I) and Hardy Weinberg (HW) testin Kaunayen chicken

Locus	Allele range (bp)	Na	Ne	I	ChiSq	Probability	Significance
HUJ 002	120-138	8	5.418	1.8736	44.435	0.025	*
MCW262	41-73	14	4.8784	1.948	56.218	0.799	ns
MCW317	225-251	6	2.9097	1.254	23.091	0.082	ns
LEI155	59-113	13	9.8567	2.4046	99.291	0.052	ns
LEI180	187-217	10	5.1206	1.8712	31.403	0.938	ns
MCW213	261-307	12	5.9024	2.081	112.961	0.000	***
MCW266	151-169	7	2.6002	1.1983	82.613	0.000	***
MCW217	147-203	9	4.1435	1.6201	84.362	0.000	***
MCW250	224-234	4	2.87	1.1769	14.224	0.027	*
MCW84	93-101	5	3.6507	1.3769	13.061	0.220	ns
LEI147	223-281	10	3.4968	1.6734	64.520	0.030	*
LEI74	287-317	9	4.2894	1.7208	40.293	0.286	ns
LEI90	200-208	5	2.6657	1.1455	37.931	0.000	***
LEI122	263-313	11	1.6565	1.0092	117.003	0.000	***
LEI82	245-275	5	3.0602	1.2596	41.463	0.000	***
LEI98	156-170	7	2.8297	1.2794	18.859	0.594	ns
MCW228	216-240	9	2.3362	1.2795	53.762	0.029	*
LEI64	261-301	11	5.4602	1.9744	103.489	0.000	***
MCW305	246-278	9	5.858	1.9137	80.961	0.000	***
LEI166	230-262	6	1.6948	0.8445	63.254	0.000	***
MCW261	226-258	10	2.8373	1.5318	87.651	0.000	***
LEI120	252-317	15	4.9087	2.0639	183.725	0.000	***
LEI174	226-256	4	2.6965	1.1359	10.920	0.091	ns
HUJ003	141-179	13	7.5014	2.223	69.728	0.737	ns
MeanSt		8.8333	4.1101	1.5775			
Dev			3.2123	1.9325	0.4261		

*P<0.05; **P<0.01; ***P<0.001; ns, nonsignificant.

domestic chicken populations maintained at the farms (White Leghorn, Aseel and Red Cornish) using 25 microsatellite markers (Kumar *et al.* 2015). The authors opined that the higher allele number may be due to the fact that most of the alleles were present in low frequency. Similarly, the effective number of alleles in indigenous poultry as reported by Tantia *et al.* (2006) was 6.27 while Kaunayen had 4.11 effective number of alleles. Pandey *et al.* (2002, 2005) reported average number of effective alleles based on 15 microsatellite loci in 3 Indian chicken populations as 4.8 (Aseel), 5.27 (Miri) and 4.27 (Nicobari), similar to that observed in the present study on Kaunayen (4.11).

The allelic diversity in Kaunayen was still higher even in comparison to local hill fowl of Uttarakhand (Na = 6.32), another non-descript population of Himalayan region of India (Phangchopi *et al.* 2014). Allelic diversity of similar magnitude was observed in indigenous Korean chicken, where number of alleles ranged from 2 to 15 per locus, with a mean of 8.13 (Suh *et al.* 2014). Much lower mean observed number of alleles have been reported in commercial broiler (4.1) as compared to desi chicken of India (8.6) (Pirany *et al.* 2007).

Genetic diversity of Kaunayen: Kaunayen poultry had substantial genetic variation based on its gene diversity in addition to the average number of alleles per locus. The observed and expected heterozygosity values ranged from 0.368 (LEI166) to 1 (MCW262) and from 0.402 (LEI122) to 0.911 (LEI155) with an overall mean of 0.664± 0.18 and 0.716±0.13, respectively (Table 2).The observed heterozygosity depends on the number of heterozygous individuals in the population and the expected heterozygosity depends on the number of alleles and their frequency in a population at a particular locus.

Average genetic variation (0.706±0.13) observed in this study was of the similar magnitude as reported for most of the other Indian breeds of poultry (Tantia *et al.* 2006). Only five breeds of indigenous poultry (Ankleswar, Kadaknath, Miri, Nicobari, Tellichery) have gene diversity less than 0.70. Heterozygosity higher than that observed in the present population (0.706) has been encountered only in Aseel (0.743), Chittagong (0.751), Danki (0.740), H Black (0.732), K Favorolla (0.739) and Kalasthi (0.724) breeds of India. In conservation programs, the maintenance of genetic diversity is the major objective so that population can face environmental challenges in the future and to respond to long term selection, either natural or artificial for traits of economic and cultural interest. The amount of heterozygosity estimated for Indian chicken is fairly large to the corresponding estimates of local Swedish chickens (Abede *et al.* 2015) and six local Italian chicken breeds (Zanetti *et al.* 2010). Whereas, heterozygosity estimates of Kaunayen were comparable with different local chicken breeds of Zimbabwe (Muchadeyi *et al.* 2007), Turkey (Kaya *et al.* 2008), Vietnam (Cuc *et al.* 2010) and Iran (Alipanah *et al.* 2011). Greater gene diversity was also reported by Zhang *et al.* (2002) in two Chinese Silkie varieties, Taihe Silkies (0.75) and Black Silkies (0.77), using nine microsatellite markers.Variation is mostly less in commercial layers and lines as compared to the local breeds and is attributed to the strong selection practiced in commercial poultry.

Observed heterozygosity was lower than expected showing a departure from Hardy-Weinberg Equilibrium (HWE) and possibility of inbreeding. Significant deviation from HWE was observed in 15 out of 24 loci at $P<0.05$ (Table 1). If a population deviates significantly from HWE at a number of independent loci, it may actually be composed of discrete demes, subject to migration from an external source or is perhaps undergoing non-random mating. Ewens-Watterson Test for Neutrality revealed that all the microsatellite markers (Table 3) were neutral as observed *F* values lie within the upper and lower limits of 95% confidence region of the expected *F* values.

Since 100% loci were neutral, selection as a cause of the decrease in observed heterozygosity was ruled out. Thus the difference between the observed and expected heterozygosity can be the non-random mating among the individuals of the population. This was also reflected in the positive F_{IS} value (0.0598) which ranged from –0.2578 to 0.4471 (Table 2). Sixty six percent of the entire set of 24 loci contributed to the overall heterozygote deficiency. Eight

Table 2. Heterozygosity statistics for all microsatellite loci in Kaunayen chicken

Locus	Sample size	Observed heter-zygosity	Expected heter-zygosity	Nei's expected* heter-zygosity	F_{IS}
HUJ 002	64	0.9062	0.8284	0.8154	-0.1114
MCW262	76	1	0.8056	0.795	-0.2578
MCW317	74	0.6486	0.6653	0.6563	0.0117
LEI155	76	0.9211	0.9105	0.8985	-0.025
LEI180	76	0.8684	0.8154	0.8047	-0.0792
MCW213	66	0.8182	0.8434	0.8306	0.0149
MCW266	74	0.5676	0.6238	0.6154	0.0777
MCW217	76	0.7632	0.7688	0.7587	-0.0059
MCW250	74	0.7568	0.6605	0.6516	-0.1614
MCW84	72	0.6111	0.7363	0.7261	0.1583
LEI147	74	0.6486	0.7238	0.714	0.0916
LEI74	68	0.6765	0.7783	0.7669	0.1179
LEI90	62	0.3871	0.6351	0.6249	0.3805
LEI122	70	0.3714	0.4021	0.3963	0.0628
LEI82	72	0.6667	0.6827	0.6732	0.0097
LEI98	72	0.6667	0.6557	0.6466	-0.031
MCW228	74	0.5946	0.5798	0.572	-0.0396
LEI64	62	0.4516	0.8302	0.8169	0.4471
MCW305	76	0.7895	0.8404	0.8293	0.048
LEI166	76	0.3684	0.4154	0.41	0.1014
MCW261	74	0.4324	0.6564	0.6476	0.3322
LEI120	68	0.6765	0.8082	0.7963	0.1505
LEI174	76	0.5263	0.6375	0.6292	0.1635
HUJ003	74	0.8108	0.8786	0.8667	0.0645
Mean	72	0.6637	0.7159	0.7059	0.0598
St. Dev		0.1799	0.1315	0.1296	0.158

*Nei (1973).

loci revealed negative F value (FÂO) indicating absence of inbreeding at these loci and some degree of out crossing in the population of Kaunayen. The observation is in agreement with that of other Indian chicken breeds as none of the chicken breeds had a negative F_{IS} value and thus out-breeding/ cross-breeding of the native chicken breeds of India has been ruled out (Tantia *et al.* 2006). Except Danki breed (F_{IS} = 0.030), all the recognized breeds of India have higher heterozygote deficiency than the Kaunayen poultry (F_{IS} =0.0598) with Tellichery having the highest (0.174).

Genetic bottleneck estimation: The majority of loci will exhibit an excess of heterozygotes in a recently bottlenecked population (exceeding the heterozygosity expected in a population at mutation drift equilibrium). Hence, to estimate the excess of such heterozygosity, Sign, Standardized differences and Wilcoxon sign rank tests were applied. The actual mutation model of evolution followed by our microsatellites is not known, thus all the three models (IAM, TPM and SMM) were selected for running the program Bottleneck. The values of average heterozygosity (He) and their probabilities (H > He) in the Sign test, under three models of microsatellite evolution were calculated and used to measure the expected number of loci with heterozygosity excess (Table 4).

Heterozygosity excess under IAM was not significantly (P>0.05) lower than the observed numbers of loci. Similarly, except for standardized difference test, heterozygosity excess under TPM was also not significantly less (P>0.05). Only under SMM, Heterozygosity excess was significant for two tests (standardized difference and Wilcoxon rank), thus the null hypothesis that the population is under mutation-drift equilibrium was accepted (Table 4). These results indicate that, due to mutation-drift equilibrium, the Kaunayen population has not undergone a recent genetic bottleneck. It has been considered that the most useful markers for bottleneck detection are those evolving under IAM, and they provide guidelines for selecting sample sizes of individuals and loci (Cornuet and Luikart 1996, Di Rienzo *et al.* 1994, Spencer *et al.* 2000); meanwhile, the TPM is thought to more closely simulate microsatellite mutation (Estoup and Cornuet 1999). Unlike the SMM, which predicts all mutations corresponding to the increment or decrement of a single base-pair repeat, the TPM predicts the occurrence of an occasional multiple base-pair repeat (Di Rienzo *et al.* 1994). The strict SMM is obviously the most conservative model for testing for a significant heterozygosity excess caused by bottlenecks, because in some conditions, it can produce a heterozygosity deficiency, and due to the heterozygosity excess it is always lower than other mutation models. Thus we have considered results from all the three tests together and it is clear that serious demographic bottlenecks have most probably not occurred in this breed.

The mode-shift indicator test was also utilized as a second method to detect potential bottlenecks, as the non-bottleneck populations that are near mutation-drift equilibrium are expected to have a large proportion of alleles with low frequency. This test discriminates many bottle necked populations from stable populations (Luikart 1997, Luikart and Cornuet 1997). A graphical representation utilizing allelic class and proportion of alleles showed a normal 'L' shaped distribution (Fig. 1).

Table 3. The Ewens-Watterson Test for Neutrality of microsatellite loci

Locus	n	k	Obs. F	Min F	Max F	Mean*	SE*	L95*	U95*
HUJ 002	64	8	0.1846	0.125	0.8052	0.2982	0.0093	0.1743	0.5356
MCW262	76	14	0.205	0.0714	0.7164	0.175	0.0029	0.1084	0.3144
MCW317	74	6	0.3437	0.1667	0.874	0.3973	0.0181	0.2195	0.7327
LEI155	76	13	0.1015	0.0769	0.7341	0.1917	0.0039	0.1163	0.367
LEI180	76	10	0.1953	0.1	0.7912	0.252	0.0071	0.1489	0.4702
MCW213	66	12	0.1694	0.0833	0.7222	0.1963	0.0035	0.1253	0.3393
MCW266	74	7	0.3846	0.1429	0.851	0.3487	0.0134	0.198	0.6647
MCW217	76	9	0.2413	0.1111	0.8116	0.2785	0.0085	0.1614	0.5218
MCW250	74	4	0.3484	0.25	0.9222	0.544	0.0259	0.3123	0.8714
MCW84	72	5	0.2739	0.2	0.8951	0.4681	0.0225	0.2585	0.8179
LEI147	74	10	0.286	0.1	0.7863	0.2515	0.0068	0.1497	0.4755
LEI74	68	9	0.2331	0.1111	0.7924	0.2746	0.0087	0.1596	0.5026
LEI90	62	5	0.3751	0.2	0.8793	0.4567	0.0223	0.257	0.7919
LEI122	70	11	0.6037	0.0909	0.7551	0.2249	0.0056	0.1367	0.4135
LEI82	72	5	0.3268	0.2	0.8951	0.4707	0.023	0.26	0.8179
LEI98	72	7	0.3534	0.1429	0.8472	0.3487	0.013	0.2037	0.6358
MCW228	74	9	0.428	0.1111	0.8072	0.2811	0.0086	0.1618	0.5091
LEI64	62	11	0.1831	0.0909	0.7294	0.2127	0.0045	0.1259	0.384
MCW305	76	9	0.1707	0.1111	0.8116	0.2822	0.0089	0.1614	0.5301
LEI166	76	6	0.59	0.1667	0.8771	0.4101	0.0187	0.2247	0.7407
MCW261	74	10	0.3524	0.1	0.7863	0.2556	0.0071	0.1479	0.4726
LEI120	68	15	0.2037	0.0667	0.673	0.1554	0.002	0.0986	0.2868
LEI174	76	4	0.3708	0.25	0.9242	0.5511	0.0289	0.2974	0.8985

Table 4. Population bottleneck analysis in Kaunayen Chicken

Model used		I.A.M.	T.P.M.	S.M.M.
Sign test (No. of loci with heterozygosity excess)	Expected	14.34	14.31	14.13
	Observed	16	10	7*
	P- value	0.31941	0.05781	0.00311
Standardized differences test	T2 value	0.528	-4.117*	-12.223*
	P- value	0.29861	0.00002	0.0000
Wilcoxon test (one tail for H excess)	P- value	0.08440	0.90625	0.99984

*Rejection of null hypothesis and bottleneck.

The L shaped curve indicated the abundance of low frequency (<0.10) alleles. This finding suggested the absence of any detectably large, recent genetic bottleneck (last 40–80 generations) in the population. Taken together all the results indicate the absence of bottleneck events in the recent past history of this breed.

Genetic relationship between Kaunayen and other chicken breeds: The genetic distance between populations provides a relative estimate of the time elapsed since the subdivisions existed as a single population and helps in characterizing the breeds or lines. Nei (1987) suggested that for construction of the topology DA and Dc are the preferred genetic distances. Thus CavalliSforzas' Edward chord distance (Dc) and Nei's standard genetic distance (DA) were estimated among the populations.

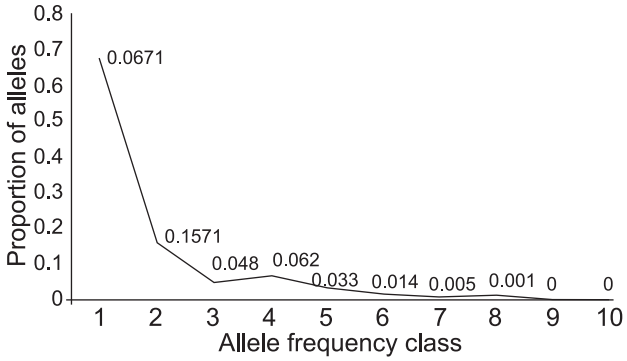

Fig. 1. Mode shift curve depicting lack of bottleneck in Kanauyen chicken.

The values for the Nei's standard genetic distance have been depicted in Table 5.

Nei's genetic distance was in the range of 0.125–1.088 (Table 5). Among the 19 pairs of populations studied, the chickens of Manipur (Kaunayen) was most distant from Red Jungle fowl and Hill fowl with a Nei's genetic distance value of 1.08. The Ghagus breed of Indian chicken was least distant with a value of 0.125. The genetic distance of such magnitude is predictable for the ecotypes which are isolated from each other for a longer number of generations and they are also subjected to differential selection pressures. The exchange of genes between populations homogenizes allele frequencies between populations and determines the relative effects of selection and genetic drift. High gene flow precludes local adaptation (i.e. the fixation of alleles, which are favoured under local conditions), and will therefore also impede the process of speciation (Balloux and Lugon-moulin 2002). CavalliSforzas' Edward chord distance (Dc) was utilized for construction of topology following Neighbour joining (NJ) algorithm (Fig. 2). Tree topology is in consonance with the geographical distribution of chicken breeds. The radiation tree places Kaunayen chicken population differently with respect to other populations. The closest is Red Jungle Fowl (Fig. 2). Most of the chicken breeds/ populations in India are isolated by distance. The same is true with Kaunayen.

Morphologically, Kaunayen look similar to Danki and

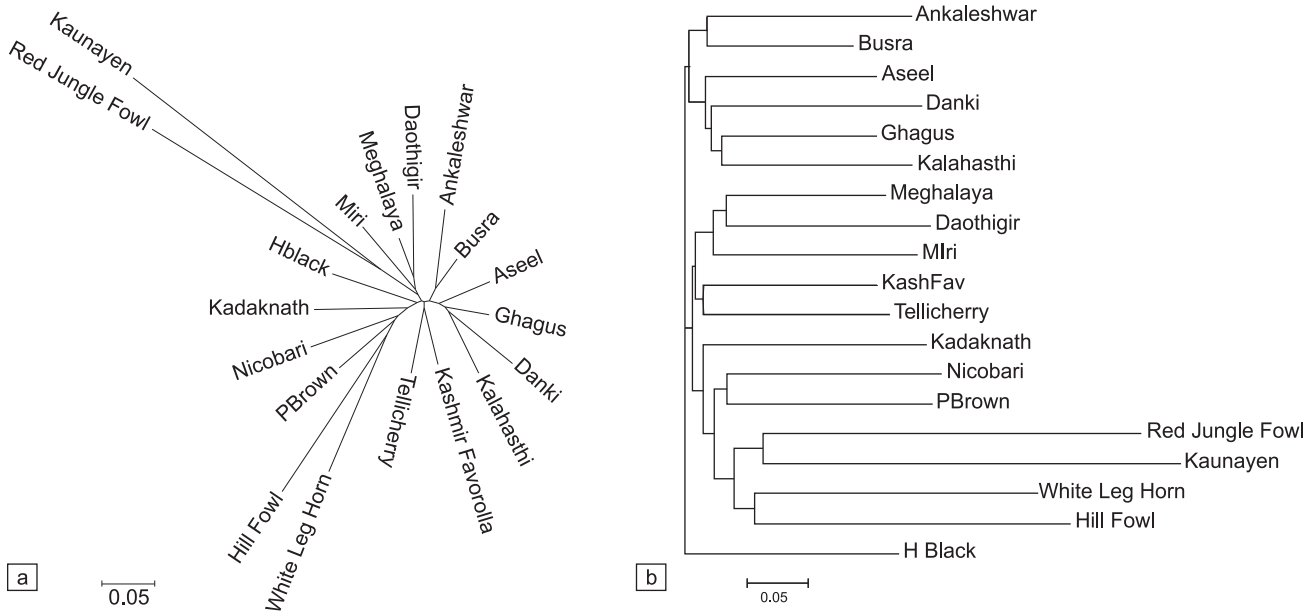

Fig. 2. Neighbor joining consensus tree constructed using Nei's(a) and Cavalli-Sforza (b) genetic distance between chicken breeds and Kaunayen population.

Aseel breeds but is isolated by distance. It is native to the area (Manipur) which is far apart and geographically isolated from the breeding tract of Danki (Andhra Pradesh) and Aseel (Andhra Pradesh, Orissa and Chhattisgarh). Over the centuries, these have adapted to the local conditions and hence can be classified as a different population of fighter birds. A similar analysis of relatedness within and among Chinese indigenous chicken using microsatellites was reported by Qu *et al.* (2006). The indigenous Chinese breeds were also highly diverse and clustered by geographic regions. Mwacharo *et al.* (2007) also used microsatellites to assess genetic diversity among African indigenous chicken from Ethiopia, Kenya, Sudan and Uganda where the populations appeared to cluster by region as could be expected from geographic (or reproductive) isolation.

In conclusion, there is sufficient evidence to suggest that the Kaunayen population that has been registered as the 17th chicken breed of India (http://www.nbagr.res.in) is genetically distinct. This breed is a reservoir of genetic diversity and should be conserved looking into its adaptive traits and socio-cultural practices of local communities rearing them.

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Table 5. Matrix of genetic distance for the Indian poultry breeds and Kaunayen population

	Ankaleshwar	Aseel	Busra	Meghalaya	Daothigir	Dunki	Ghagus	HBLack	Kadaknath	Kalahasthi	Kashmir Favorolla	Miri	Nicobari	Punjab Brown	Tellicherry	Red Fowl Jungle	White Leg Horn	Hill Fowl	Kaunayen
Ankaleshwar	0	0.337	0.289	0.343	0.379	0.385	0.336	0.364	0.367	0.367	0.345	0.382	0.416	0.395	0.362	0.552	0.5	0.512	0.588
Aseel	0.216	0	0.276	0.300	0.367	0.323	0.284	0.348	0.361	0.304	0.317	0.336	0.372	0.385	0.310	0.535	0.464	0.503	0.557
Busra	0.16	0.138	0	0.312	0.354	0.348	0.291	0.314	0.323	0.329	0.289	0.336	0.362	0.347	0.295	0.522	0.451	0.484	0.569
Meghalaya	0.227	0.151	0.182	0	0.299	0.362	0.321	0.355	0.353	0.329	0.302	0.309	0.363	0.349	0.331	0.514	0.446	0.482	0.539
Daothigir	0.322	0.298	0.275	0.17	0	0.409	0.361	0.386	0.360	0.393	0.353	0.348	0.394	0.365	0.359	0.549	0.441	0.503	0.576
Dunki	0.416	0.264	0.284	0.348	0.464	0	0.316	0.336	0.402	0.329	0.348	0.379	0.422	0.430	0.363	0.545	0.480	0.483	0.567
Ghagus	0.217	0.135	0.156	0.181	0.277	0.236	0	0.338	0.352	0.284	0.308	0.341	0.386	0.355	0.313	0.538	0.460	0.481	0.558
HBLack	0.341	0.269	0.217	0.265	0.376	0.214	0.25	0	0.379	0.367	0.335	0.371	0.374	0.366	0.353	0.530	0.427	0.469	0.584
Kadaknath	0.267	0.237	0.177	0.211	0.262	0.367	0.187	0.271	0	0.409	0.345	0.389	0.373	0.369	0.360	0.572	0.454	0.449	0.615
Kalahasthi	0.282	0.185	0.219	0.227	0.341	0.242	0.125	0.298	0.324	0	0.337	0.363	0.410	0.401	0.364	0.565	0.502	0.510	0.552
KashmirFavorolla	0.288	0.19	0.167	0.198	0.268	0.298	0.148	0.249	0.207	0.209	0	0.336	0.357	0.351	0.296	0.529	0.434	0.473	0.565
Miri	0.268	0.179	0.169	0.164	0.252	0.33	0.171	0.295	0.247	0.226	0.213	0	0.391	0.385	0.327	0.552	0.469	0.506	0.569
Nicobari	0.448	0.294	0.298	0.253	0.306	0.383	0.299	0.287	0.28	0.348	0.249	0.303	0	0.344	0.349	0.559	0.434	0.458	0.598
PunjabBrown	0.436	0.333	0.346	0.227	0.269	0.46	0.286	0.338	0.308	0.355	0.304	0.308	0.224	0	0.371	0.552	0.405	0.464	0.576
Tellicherry	0.272	0.168	0.152	0.201	0.297	0.301	0.141	0.288	0.24	0.197	0.149	0.187	0.263	0.31	0	0.532	0.426	0.486	0.572
Red Jungle Fowl	0.863	0.708	0.662	0.615	0.754	0.691	0.708	0.647	0.86	0.776	0.669	0.711	0.75	0.753	0.643	0	0.581	0.605	0.654
White Leg Horn	0.752	0.486	0.503	0.451	0.443	0.486	0.486	0.386	0.512	0.548	0.413	0.508	0.361	0.362	0.392	0.659	0	0.490	0.625
Hill Fowl	0.736	0.632	0.579	0.545	0.537	0.477	0.52	0.488	0.508	0.623	0.503	0.566	0.458	0.473	0.52	0.915	0.474	0	0.638
Kaunayen	0.856	0.71	0.796	0.677	0.859	0.762	0.761	0.761	1.009	0.673	0.847	0.753	0.96	0.791	0.82	1.083	1.008	1.088	0

Upper triangle presents Cavalli-Sforza and Edwards distance (1967) and lower triangle presents Nei's distance (1972).

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