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# Hazra chicken: A precious germplasm in need of immediate scientific intervention

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ABSTRACT

Indian poultry diversity is still largely unexplored, even though more than half of the germplasm is endangered. The present study was planned to ascertain the genetic diversity of local poultry population of Odisha (Hazra) using 25 Simple Sequence Repeat markers. All the loci were retained for diversity analysis due to their behavior as neutral markers in this population. Hazra chicken population was found to host a very high level of diversity. This conclusion is based on the large number of alleles observed across loci (average14.96, range 6–21), and by the high expected heterozygosity (average 0.80, range 0.50–0.92). In spite of high within-breed variation, signatures of inbreeding were detected by the $F_{IS}$ index, which was positive (0.218±0.03) in the population. Hazra chicken population presented the highest heterozygote deficiency as compared to all the 17 recognized poultry breeds of India. Nonsignificant heterozygote excess on the basis of infinite allele model (IAM) along with a normal 'L'-shaped distribution of mode-shift analysis test, indicated an absence of bottleneck. In summary, molecular data conclude that Hazra population possesses a very interesting pool of chicken genetic resources due to their high genetic diversity. Increased level of inbreeding is indicating that flock management and reproduction strategies deserve attention.

**Key words**: Bottleneck, Genetic diversity, Hazra chicken, Heterozygote deficiency, Indian poultry, Microsatellite markers

India is a reservoir of diverse chicken germplasm including 17 well documented and registered breeds and many more populations that have not been characterized so far (http://www.nbagr.res.in).Loss of diversity is a major concern especially in poultry where over half the breeds of the 5 major species (chicken, domestic duck, Muscovy duck, goose and turkey) are thought to be endangered or critical (FAO 2007). The approach for conservation should be based on prioritization of the concerned populations on the basis of the subset of total diversity harboured by them. Diversity needs to be determined using suitable decision support system such as molecular genetic information to judiciously utilize the limited financial resources. Therefore, the genetic resources which are yet to be evaluated and characterized are getting priority now a days.

Backyard poultry production comprising mainly of desi (indigenous) chickens provides livelihood security to the owner family in addition to securing the availability of food. The birds require no scientific feeding, health care, housing and management, and sustain on their own in low or no input conditions. The bird is acceptable to the local inhabitants since it suits their socio-cultural beliefs. One such population is Hazra of Odisha state in India. These are concentrated in the districts of north Odisha bordering the states of Jharkhand and West Bengal. The climate of these areas is hot and humid with minimum temperature of 11.5°C and maximum temperature of 31.5°C. Birds are of various colours such as dark red, black and spotted (black and white). Based on the colour pattern these are referred by local names such as *Rangia, Kaila, Ghinjira* and *Dhalua*. Red colour comb and red colour ear lobes are very common in these birds (Fig. 1). The number of eggs laid down by an adult hen in one clutch ranges from 5 to 15 with egg weight ranging from 48 to 76 g per egg (Barik 2016). The owners of Hazra birds are mostly marginal or landless farmers. The individual household keeps 5 to 50 birds of different age groups. The birds are used for meat and game (cock fighting), thereby contributing to the economy as well as nutritional security of the people of this area. Still, the population is not recognized as a distinct breed and no information is available on the existing diversity status of this population.

The exploitation of DNA polymorphism as molecular markers has opened many vistas in genetic characterization, conservation, improvement and molecular evolution studies in livestock species. The marker system that is contributing most in diversity studies are microsatellites/SSRs due to their highly polymorphic nature, abundance in the genome

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and ease of amplification by PCR (Sharma *et al.* 2013, 2015). Microsatellites or SSRs (simple sequence repeats) are now one of the most widely used molecular markers in several eukaryotic species, including birds especially due to their relative ease of scoring and ability to exhibit high levels of polymorphism (Pandey *et al.* 2005, Alipanah *et al.* 2011). Several studies have been conducted to assess chicken genetic diversity using microsatellite markers (Kaya *et al.* 2008, Wilkinson *et al.* 2011, Suh *et al.* 2014, Abebe *et al.* 2015). Also, the microsatellite loci are probably the best markers currently available for detecting recent bottlenecks because of their generally high level of variability.

In this study, 25 microsatellite markers have been used to evaluate the genetic diversity of Hazra chicken and the generated data has been utilized for estimating any recent genetic bottleneck suffered by this population. The extent of heterozygote deficiency in the population was also examined to facilitate the strategic planning of current and future breeding programs.

## MATERIALS AND METHODS

*Sampling:* Sample collection was carried out according to FAO recommendations, selecting a few birds per flock across traditional rearing area. Birds (40) were sampled from the distribution area which lies between 21°.17' and 22°.34' north latitude and 85°.40' and 87°.10' east longitude comprising 3 districts (Mayurbhanj, Keonjhar and Deogarh). 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 1 mile apart.

Fig. 1. Representative birds of Hazra chicken.

*DNA extraction, quantification and amplification:* Genomic DNA was extracted from 2 ml of blood collected from the brachial vein of the adult birds using phenol chloroform DNA isolation method.DNA integrity and quantity was checked on 1% agarose gel by direct comparison with a standard marker as well as spectrophotometrically (Nano Drop Spectrophotometer).

A panel consisting of 25 microsatellite markers was selected for the diversity analysis of Hazra population. These were chosen from literature aiming to analyze highly polymorphic markers spread across the genome. Moreover, these primers have previously been used for indigenous chicken diversity studies (Tantia *et al.* 2006). 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). 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 5 min; 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 (0.5mg/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 using LIZ 500 as the internal size standard. 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.

*Data analysis:* 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). Bottleneck events in the population were tested by 3 methods. The first method consisted of 3 excess heterozygosity tests developed by Cornuet and Luikart (1996): (i) Sign test, (ii) Standardized differences test, and (iii) Wilcoxon sign-rank test. The probability distribution was established using 1000 simulations under 3 models–Infinite allele model (IAM), step-wise 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 2 methods were applied using Bottleneck v1.2.02 (http://www.ensam.inra.fr/URLB).

## RESULTS AND DISCUSSION

The primary objective of a conservation programme is to preserve as much genetic diversity as possible (Boettcher *et al.* 2010). The present study aimed to genetically characterize a lesser known poultry population of Odisha using 25 microsatellites (SSR) amplified in multiplex PCR.

*Genetic variability of microsatellite loci and allelic*

*distribution:* All the markers were polymorphic and a total of 374 alleles were detected across the 25 loci. 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 Hazra poultry was evident from the allele frequency data (available on request). LEI174 and LEI82 showed the highest number of observed alleles per locus (21) while LEI166 showed the lowest (6) with the mean number of alleles (MNA) of 14.960 (Table 1). Expected number of alleles varied from 1.990 (LEI166) to 12.488 (LEI147) with the mean of 6.07. 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 25 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 0.910 (LEI166) to 2.701 (LEI147). 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 number of alleles at different marker loci and their frequencies are simple indicators of the population structure and its breeding history. The results suggested existence of enough genetic variation in the Hazra population for further breeding programs. The number of alleles and allele size range observed in the present investigation (Table 1) were higher as compared to that reported in 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. In subsequent study by Tantia *et al.* (2006), much higher genetic variation has been reported in the registered breeds of chicken in India (15.88). It is evident from the mean number of alleles (14.96) observed in the present study that Hazra population has sufficient genetic variation. Observed number of alleles ranging from 5 (MCW111) to 43 (LEI212) with an average number of 19 alleles per locus has been reported in Indian Red jungle fowl from northern India and 3 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. Similar can be the case of Hazra chicken population. The

Table 1. Observed (Na) and effective number of alleles (Ne), Shannon's information index (I) and allele size range in Hazra chicken

| Locus | N | Na | Ne | I | Allele size range (bp) |
|---|---|---|---|---|---|
| LEI74 | 33 | 12.000 | 4.382 | 1.847 | 283-313 |
| LEI122 | 33 | 12.000 | 3.377 | 1.709 | 267-311 |
| LEI174 | 33 | 21.000 | 6.205 | 2.409 | 220-280 |
| HUJ002 | 36 | 13.000 | 5.301 | 2.079 | 100-136 |
| LEI166 | 37 | 6.000 | 1.990 | 0.910 | 148-256 |
| MCW305 | 31 | 7.000 | 4.013 | 1.585 | 252-264 |
| LEI120 | 36 | 19.000 | 8.938 | 2.557 | 252-326 |
| LEI82 | 38 | 21.000 | 5.309 | 2.322 | 225-287 |
| LEI 155 | 32 | 13.000 | 2.158 | 1.399 | 71-115 |
| MCW 213 | 30 | 15.000 | 9.890 | 2.452 | 263-323 |
| MCW228 | 36 | 18.000 | 5.864 | 2.280 | 202-250 |
| MCW262 | 38 | 18.000 | 9.890 | 2.542 | 43-91 |
| MCW266 | 35 | 12.000 | 5.671 | 2.043 | 149-173 |
| MCW317 | 38 | 20.000 | 7.912 | 2.452 | 203-269 |
| LEI147 | 32 | 19.000 | 12.488 | 2.701 | 225-301 |
| MCW250 | 30 | 15.000 | 8.182 | 2.360 | 210-250 |
| MCW176 | 36 | 17.000 | 8.308 | 2.409 | 240-274 |
| MCW261 | 36 | 13.000 | 7.784 | 2.240 | 214-258 |
| LEI98 | 36 | 14.000 | 5.423 | 2.068 | 142-168 |
| LEI90 | 34 | 19.000 | 6.701 | 2.376 | 182-226 |
| MCW217 | 37 | 18.000 | 3.928 | 2.021 | 131-197 |
| MCW84 | 35 | 10.000 | 2.495 | 1.344 | 73-101 |
| LEI64 | 33 | 14.000 | 4.735 | 1.973 | 261-307 |
| LEI180 | 36 | 11.000 | 5.014 | 1.938 | 167-207 |
| HUJ003 | 36 | 17.000 | 5.773 | 2.234 | 121-191 |
| Mean | 34.680 | 14.960 | 6.069 | 2.090 | |
| SE | 0.486 | 0.824 | 0.522 | 0.086 | |

Na, No. of different alleles; Ne, No. of effective alleles, 1 / (Sum pi^2); I, Shannon's information index, -1* Sum (pi * Ln), where pi is the frequency of the ith allele for the population and Sum pi^2 is the sum of the squared population allele frequencies.

effective number of alleles in registered breeds of indigenous poultry as reported by Tantia *et al.* (2006) was 6.27 which is comparable to that observed in Hazra population (6.069). Much lower number of effective alleles has been reported by Pandey *et al.* (2002) in 3 Indian chicken populations viz. 4.8 (Aseel), 5.27 (Miri) and 4.27 (Nicobari).

The allelic diversity in Hazra is 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 lower magnitude has also been reported 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).

*Gene diversity:* 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. Hazra 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 0.355 (MCW305) to 0.895(MCW262) and from 0.497 (LEI166) to 0.920 (LEI147) with an overall mean of 0.619± 0.03 and 0.796±0.02, respectively (Table 2).

Average genetic variation (0.796±0.02) 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 5 breeds of indigenous poultry (Ankleshwar, Kadaknath, Miri, Nicobari, Tellichery) have gene diversity less than 0.70.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 6 local Italian chicken breeds (Zanetti *et al.* 2010). Whereas, heterozygosity estimates of Hazra were comparable with different local chicken breeds of Zimbabwe (Muchadeyi *et al.* 2007), Turky (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 2 Chinese Silkie varieties, Taihe Silkies (0.75) and Black Silkies (0.77), using 9 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 all the loci at P< 0.001 (Table 2). 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 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

Table 2. Summary of heterozygosity statistics for all loci in Hazra chicken with Hardy Weinberg (HW) tests

| Locus | N | Ho | He | UHe | F | ChiSq | Probability | Significance |
|---|---|---|---|---|---|---|---|---|
| LEI74 | 33 | 0.636 | 0.772 | 0.784 | 0.175 | 102.163 | 0.003 | ** |
| LEI122 | 33 | 0.727 | 0.704 | 0.715 | -0.033 | 130.621 | 0.000 | *** |
| LEI174 | 33 | 0.636 | 0.839 | 0.852 | 0.241 | 404.517 | 0.000 | *** |
| HUJ002 | 36 | 0.667 | 0.811 | 0.823 | 0.178 | 168.569 | 0.000 | *** |
| LEI166 | 37 | 0.432 | 0.497 | 0.504 | 0.131 | 113.497 | 0.000 | *** |
| MCW305 | 31 | 0.355 | 0.751 | 0.763 | 0.527 | 59.873 | 0.000 | *** |
| LEI120 | 36 | 0.528 | 0.888 | 0.901 | 0.406 | 361.697 | 0.000 | *** |
| LEI82 | 38 | 0.789 | 0.812 | 0.822 | 0.027 | 382.280 | 0.000 | *** |
| LEI 155 | 32 | 0.438 | 0.537 | 0.545 | 0.185 | 127.913 | 0.000 | *** |
| MCW 213 | 30 | 0.800 | 0.899 | 0.914 | 0.110 | 166.316 | 0.000 | *** |
| MCW228 | 36 | 0.694 | 0.829 | 0.841 | 0.163 | 281.380 | 0.000 | *** |
| MCW262 | 38 | 0.895 | 0.899 | 0.911 | 0.005 | 277.881 | 0.000 | *** |
| MCW266 | 35 | 0.571 | 0.824 | 0.836 | 0.306 | 146.418 | 0.000 | *** |
| MCW317 | 38 | 0.737 | 0.874 | 0.885 | 0.157 | 359.055 | 0.000 | *** |
| LEI147 | 32 | 0.719 | 0.920 | 0.935 | 0.219 | 270.244 | 0.000 | *** |
| MCW250 | 30 | 0.500 | 0.878 | 0.893 | 0.430 | 201.546 | 0.000 | *** |
| MCW176 | 36 | 0.611 | 0.880 | 0.892 | 0.305 | 327.997 | 0.000 | *** |
| MCW261 | 36 | 0.667 | 0.872 | 0.884 | 0.235 | 135.856 | 0.000 | *** |
| LEI98 | 36 | 0.556 | 0.816 | 0.827 | 0.319 | 150.959 | 0.000 | *** |
| LEI90 | 34 | 0.559 | 0.851 | 0.863 | 0.343 | 407.871 | 0.000 | *** |
| MCW217 | 37 | 0.568 | 0.745 | 0.756 | 0.239 | 225.877 | 0.000 | *** |
| MCW84 | 35 | 0.571 | 0.599 | 0.608 | 0.046 | 87.103 | 0.000 | *** |
| LEI64 | 33 | 0.545 | 0.789 | 0.801 | 0.308 | 188.241 | 0.000 | *** |
| LEI180 | 36 | 0.778 | 0.801 | 0.812 | 0.028 | 144.325 | 0.000 | *** |
| HUJ003 | 36 | 0.500 | 0.827 | 0.838 | 0.395 | 224.721 | 0.000 | *** |
| Mean | 34.680 | 0.619 | 0.796 | 0.808 | 0.218 | | | |
| SE | 0.486 | 0.026 | 0.022 | 0.022 | 0.029 | | | |

Ho, Observed heterozygosity = No. of Hets / N; He, Expected heterozygosity = 1 - Sum pi^2; UHe, Unbiased expected heterozygosity = (2N / (2N-1)) * He; F, Fixation index = (He - Ho) / He = 1 - (Ho / He); Where pi is the frequency of the ith allele for the population and Sum pi^2 is the sum of the squared population allele frequencies; **P<0.01; ***P<0.001.

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 (0.218) which ranged from –0.033 to 0.527 (Table 2). All the loci except LEI122 contributed to the overall heterozygote deficiency. The $F_{IS}$ measures the reduction of heterozygosity because of nonrandom mating within the population. Hence F value greater or lesser than zero reveals inbreeding or out breeding.

The observation is in unison with that of recognized Indian chicken breeds as none of the chicken breeds had a negative $F_{IS}$ value and thus out-breeding/ crossbreeding of the native chicken breeds of India has been ruled out (Tantia *et al.* 2006). Tellichery breed has the highest (17.4%) heterozygote deficiency among the 17 recognized breeds of India. However, Hazra chicken population has even higher (21.8%) heterozygote deficiency. The strategy of sampling very few birds per flock in the current study was aimed at minimizing the artificial substructuring of the samples, the associated Wahlund effect as well as the inclusion of related animals. Therefore, the observed results seem to reveal real inbreeding in Hazra population. Small flock size and ignorance of reproductive strategies are causing undesirable rates of inbreeding in the population. This trend should be properly monitored by governmental and non-governmental organizations involved in promoting the development of animal husbandry in Odisha.

*Genetic bottleneck analysis:* In recently bottlenecked populations, the majority of loci will exhibit an excess of heterozygotes, thus exceeding the heterozygosity expected in a population at mutation drift equilibrium. To estimate the excess of such heterozygosity Sign, Standardized differences and Wilcoxon sign rank tests were utilized. The actual mutation model of evolution followed by our microsatellites is not known, thus all the 3 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 3 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 except for standardized difference test. However, except for Wilcoxon test heterozygosity excess under TPM was significantly less (P>0.05). Similarly under SMM, heterozygosity excess was significant for 2 tests (Sign and standardized difference). Thus the null hypothesis that the population is under mutation-drift equilibrium was questionable. 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 occasonal 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 3 tests together.

It is not clear whether any serious demographic bottlenecks have occurred in this population, hence the Mode-shift indicator test was also utilized as a second method to detect potential bottlenecks. 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. 2). 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 indicated the absence of bottleneck events in the recent past history of this breed.

In conclusion, characterization and conservation of populations such as Hazra is necessary, looking into its

Table 4. Population bottleneck analysis for Hazra chicken

| Model used | | I.A.M. | T.P.M. | S.M.M. |
|---|---|---|---|---|
| Sign test (No. of loci with hetrozygosity excess) | Exp | 15.17 | 14.92 | 14.70 |
| | Obs | 11 | 5* | 2* |
| | P- value | 0.0678 | 0.0001 | 0.0000 |
| Standardized differences test | T2 value | -3.229* | -10.653* | -21.572* |
| | P- value | 0.0006 | 0.0000 | 0.0000 |
| Wilcoxon test (one tail for H excess) | P- value | 0.9463 | 1.0000 | 1.0000 |

*Null hypothesis that population is in under mutation-drift equilibrium is rejected.

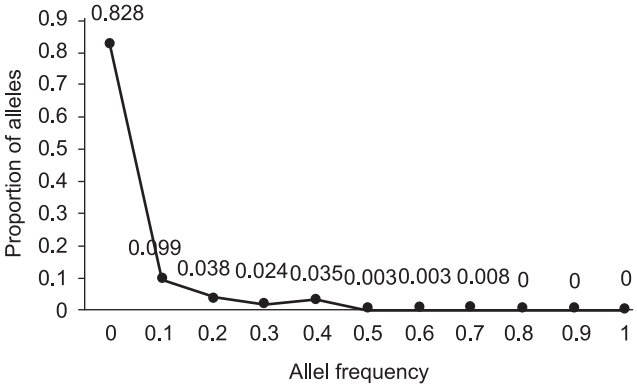


Fig. 2. Graphic representation of proportion of alleles and their distribution in Hazra chicken.

adaptive traits and socio-cultural practices of local communities rearing them.High heterozygosity and polymorphism indicated abundance of genetic variation in this native chicken population. However, there were indications that variability is being lost as shown by highest degree of heterozygote deficiency recorded in Hazra among the Indian chicken. It is suggested that Hazra cocks should be exchanged among the communities to increase the heterozygosity.Population available in nearby areas can also be used for breeding. The information generated from the present study is based on the microsatellite markers that have previously been used for indigenous chicken diversity studies (Tantia *et al*. 2006). Hence the data can be compared with that of seventeen registered Indian poultry breeds to establish its distinction from the catalogued gene pool.

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