Estimation of Genetic Diversity between an Indigenous: Kadaknath and Commercial White Leghorn breeds of Chicken by using STR Markers

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ABSTRACT

Complexity of Biological data can be resolved out through advent tools of computational statistical and Molecular analysis to drawn reliable estimates for the estimation of Genetic diversity. The present study was conducted to evaluate intra and inter-breed genetic variation between two diverse chicken populations: Kadaknath (KN) and White Leghorn (WLH) at molecular level, using twelve highly-polymorphic microsatellites markers. Results from computational statistical analysis revealed distinctly-different population parameters; PIC, Na, Ne, Nei's index, Ho, He and Shannon's index (I), showing significantly-higher values for KN as opposed to WLH, while lower values of *F-statistics estimates* ($F_{IS,}$ F_{ST} and F_{IT}) were recorded for KN as compared to WLH. Intra-breed variability assessed through un-rooted dendrogram generated for these populations *via* neighbor-joining algorithm exhibited distinctly-different dispersal pattern by higher inter-sample divergence in KN than WLH. It could be inferred that variability of WLH appeared eroded over generations due to operational evolutionary-force (selection) while KN appeared to retain more heterozygosity consistent to its breeding history.

Key Words: Chickens, Conservation, Diversity, Kadaknath, Microsatellites, Selection etc.

1. INTRODUCTION

Traditionally, inter-breed genetic differences between organized chicken populations are based on quantitative analysis using genetic and phenotypic parameters for various economic traits. However, quantitative genetic approaches are fraught with various limitations like: need for structured-pedigrees, information from sibs and robust statistical designs, which reduce their applications in breeding programs. With advent tools of DNA markers system, understanding of inherent diversity within families, species and between populations of chicken breeds has simplified to a great extent. Assessing the genetic diversity among chicken breeds by using molecular tools is also essential for designing future conservation and genetic improvement programmes (Osman *et al*., 2006). On the basis of these informations appropriate strategies could be formulated for the conservation of various chicken breeds, including the indigenous ones that harbor several unique alleles but remain under threat of extinction leading to permanent loss of valuable genotypes and traits.

Among available DNA markers, microsatellites which also known as short tandem repeats (STRs) are most reputed markers of choice, as they provide a polymorphic and robust marker system, being abundant, co-dominant, randomly available across genome, having high information content due to variable number of repeats, high mutation rate, ability to decipher

moderate to high level of variability, amenability to PCR and ease of genotyping (Pandey *et al*., 2005; Kaya and Yildiz, 2008 and Pratap *et al*., 2012).
Microsatellite markers have been successfully used in many studies of genetic diversity in chickens (Romanov and Weigend, 2001). The microsatellite loci represent an independent evolutionary history of a population if they fulfill the conditions like Mendelian inheritance; reasonable PIC values; presence on different chromosomes/linkage groups and independent assortment (Rajkumar *et al*., 2008). The objective of current study was to analyze intra and inter-breed genetic diversity on molecular level between a prominent native chicken breed: Kadaknath (KN) *vis a vis* a popular White Leghorn strain (WLH) maintained with distinctly-different breeding-regime at CARI, India under proper healthcare and mannagemental conditions by using a panel of polymorphic microsatellites. Since these breeds are phenotypically and genetically distinct, their differential molecular-analysis of genetic variations may facilitate understanding of their inherent mode of genetic diversity.

2. MATERIAL AND METHODS

Current study utilized two distinctly-diverse chicken populations: Kadaknath (KN) *vis a vis* a long term selected White Leghorn (WLH), bred at CARI, Izatnagar, India, which were not only different by geographic origin, but also had contrasting breeding histories. The breeding history of KN revealed that this was picked up from its home tract Central India (spreading over Jhabua and Dhar districts of Madhya Pradesh) in late seventies and since then conserved at CARI, as a closed population. KN has been maintained under pedigreed random-mating (with adequate sire and dam family base), with no-deliberate selection, while emphasis is laid on undiluted genetic diversity. The KN is only second internationally-documented reputed native breed hosting 'Fibromelanosis' traits (next to Silkie from China) that renders hyperpigmentation in the skin and visceral organs, caused due to the existence of 'Fm' gene fixed in this population (Mishra *et al*., 2008). In contrast, WLH strain: Izatnagar White Leghorn (IWH) was introduced into India (CARI) during the year 1972 from USA and was maintained as a closed flock (Singh and Sharma, 2002). Since then, WLH has been the bred of productivity through continued selection regimen by employing an Individual-dam-sire (IDS) family selection index (Osborne, 1957) for high egg number from more than 30 generations, in which initial 22 selection cycles were for part period egg production (till 40 weeks of age) and the remaining 8 cycles utilized whole-year egg production (till 64 weeks of age).

3. DNA EXTRACTION AND PCR GENOTYPING

DNA was extracted from 36 randomly selected hens from each breed by using a standard Phenol-chloroform protocol (Sambrook and Russel, 2001) and thereafter subjected to standard microsatellite-PCR amplification as per Pratap, (2011). The individual PCR reactions were carried out with appropriate PCR thermal cycling conditions. Microsatellites were picked up from Kit#7 designed by genome-mapping lab, MSU, East Lansing, USA especially for uniform inter-marker spacing across chicken genome.
Total 32 microsatellites were typed which gave distinct allelic patterns for the interpretation in current study, out of which only twelve highly-polymorphic loci were used for further analysis. The confirmation of PCR reactions and alleles size was carried out by using a high resolution Metaphor-agarose (Lonza Inc., Rockland ME, U.S.A) electrophoresis with a sufficient gel migration for better resolution. The exact sizing of STR alleles was accomplished by using an

ABI Automated Sequencer (Applied Bio system^{Tm}, ABI-3130 facilities at Chromous Biotech (P) Ltd. Bangalore).

4. STATISTICAL ANALYSIS

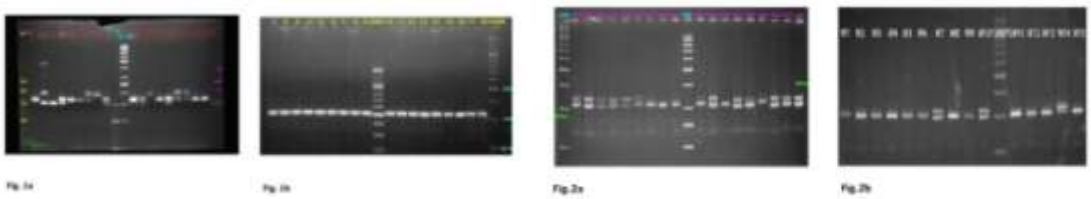
The genotyping data was analyzed with computational statistical softwares; POP-Gene, Gene-Alex, F-*STAT* and MS-Tool Kit to analyze various population parameters included: observed heterozygosity (Ho), effective number of alleles (Ne), allele frequencies, Nei's unbiased heterozygosity, Shannon's Information Index (I), Polymorphism Information Content (PIC) and *F-statistics* estimates (F_{IT}, F_{ST} and F_{IS}). The dispersion pattern among respective DNA samples within and between the populations was studied by Phylogenetic analysis was carried out by POP-Gene, using Neighbour-Joining (NJ) algorithm (Saitou and Nei, 1987).

5. RESULTS AND DISCUSSION

Various parameters of genetic diversity with respect to 12 polymorphic STRs for both chicken breeds are summarized in Table1. Out of tested 12 STRs, ten loci were heterozygous for both populations, while remaining two loci showed isomorphism in WLH but segregated in the KN. STRs employed in our study yielded higher PIC score for KN (0.54) as opposed to WLH (0.32). The PIC refers to the values of the marker for detection of polymorphism which depend upon number of detectable alleles, their distribution and frequency at a particular locus. Mean PIC value of 0.59 was observed by Ahlawat *et al.* (2007) for multiple Indian-native chicken populations, while 0.62 was scored for Ankleshwar breed by Pandey *et al.* (2005). However, Rajkumar *et al.* (2008) have reported a wider range of PIC values, varying from 0.39 (in Dahlem Red) to 0.71 (in non-descript Desi breeds). Similarly, Kaya *et al.* (2008) reported PIC value of 0.599 and 0.426 for Denizli and Gerze chickens, respectively. Thus, observed PIC values for KN indicated a significant higher level of heterozygosity as compared to WLH, which was due to presence of lower number of alleles in WLH across examined loci.

The total number of alleles summed over both populations was 73 with an average of 6.08 per locus. The average number of Alleles were recorded higher (3.58 ±1.08) in KN than those of WLH (2.50 ± 1.16). Average number of alleles (Na) at a single locus in a single population would normally range from one (monomorphic) or more (polymorphic) in number (Emara *et al*., 2002).

Figures:



The gel images depicting variable alleles pattern scored for KN and WLH for Locus ADL0202 and LEI0 74 are presented in figures 1(a and b) and 2 (a and b) respectively which are self-revealing on the relative abundance of alleles in KN over WLH .

The number of alleles (Na) across both populations were recorded 2 to 5 in KN and 1 to 4 in WLH is also supported by various reports; 2.8 to 2.9 by Croojimans *et al*. (1996); 2.5 to 3.5 by

Emara *et al*. (2002); 3.6 by Nasiri *et al*. (2007); 5 to 6 by Kaya *et al*. (2008) and 3.8 by Liu *et al*. (2008). At the same time, higher Na for two similar populations like ours: Kadaknath and White Leghorn (8.59 and 8.448 respectively) has been documented by Ahlawat *et al*. (2007), and even further-high Na values have been recorded by some authors, i.e. 8.6 for randomly sampled local chickens (non-descript ones) by Pirany *et al.* (2007) and 9.55 for Chinese native chicken by Chen *et al.*(2008). However, it may be noted that higher Na values as reported by the above authors have only come from the field samples of native chickens including the KN samples (Ahlawat *et al*., 2007) unlike the closed flock of KN investigated in our study.

The mean effective number of alleles (Ne) values was recorded as 2.80 ± 1.03 for KN and 1.89 ±0.89 for WLH chickens. The effective number of alleles (Ne) is a nonlinear function of the He (of a population) which gives an idea about: how wide is the allele frequency-distribution in the population. The lower number of effective alleles than the observed number of alleles across most of the STRs used in present investigation indicated that allele frequency-distribution was wide enough in both populations. However, lower values in WLH in contrast to higher frequency in KN realized in our study could be due to the selection programme carried out in the former and not in the KN. Akin to our observations, Rajkumar *et al*. (2008) reported lower Ne (2.69) for Dahlem Red breed (undergoing selection) and higher Ne (4.15) for non-descript native chickens. However, Pirany *et al*. (2007) have recorded lower Ne estimates (2.7) in commercial layers than the randomly-chosen local breed (4.7). Equivalent Ne values like ours have been reported by Pandey *et al.* (2005) and Nasiri *et al*. (2007) in indigenous chicken breeds.

The observed heterozygosity (Ho) which is a state of individual possessing different alleles at a particular locus and also provides a measure of genetic diversity in a population remained higher (0.50 ± 0.17) for KN than those of WLH (0.27±0.23). Kaya *et al*. (2008) observed similar Ho values (0.508 ±0.037) in Denizli breed like that of KN, while lower values (0.38 ±0.056) were realized in Gerze breed. However contrary to our findings, higher Ho value (0.728) for a WLH population than that of KN (0.653) has been reported by Ahlawat *et al*. (2007), where the authors had sampled many chicken breeds including WLH chickens from the field, from larger area of breeding-tracts of India. This would mean that their sampling of WLH was not from a closed population like that of ours and was based on random WLH chickens including commercial ones, which were most likely three or four way crosses (as marketed by most commercial companies) and this might have given rise to higher Ho values in their flock. Moderate to high Ho values for many native breeds have been reported by earlier workers; 0.527 by Pandey *et al*. (2005), 0.5613 by Nasiri *et al*. (2007), 0.630 by Pirany *et al*. (2007), 0.422 by Liu *et al*. (2008), 0.73 by Rajkumar *et al.* (2008) and 0.538 by Davila *et al*. (2009) in different chicken breeds.

The Expected Heterozygosity (He) is an indicator of differences in adaptative conditions, geographical region, sample size, sources and reproducibility of microsatellite markers, ranged from 0.50 to 0.813 and 0.106 to 0.722 in KN and IWH respectively. Similar wide ranging estimates for He have been reported by other workers including 0.5 each by Nasiri *et al*. (2007) and Liu *et al.* (2008); 0.6 each by Romonov and Weigend (2001), Kong *et al.*,(2006), Shahbazi *et al*. (2007) and Kaya *et al*. (2008).

The mean He (0.60) of our KN flock was ably supported by other report (0.741) for the same breed by Ahlawat *et al*. (2007). However, very high value for He (0.774) has been reported for the WLH samples by the same authors, which is in variance to our findings. Pirany *et al.* (2007) on the other hand, have reported 0.52 as the He value for some commercial Layer samples (WLH). The reasons for reporting higher He values than ours (0.37, realized in our closed flock),

could again be attributed to the randomly-sampled commercial WLH chickens from the field by these authors.

A total of 14 common alleles and 44 private alleles (28 for KN and 16 for IWH) along with their respective frequencies observed in these populations, depicted in table 1. Among private alleles, the ADL0202 and MCW0005 yielded the maximum number (4 each) in KN and WLH respectively. Likewise, single private alleles were scored for ADL278 and ADL114 loci in KN and for ADL145, ADL0278, ADL0034 and ROS0302 in WLH. STR results also provided many discernible markers which differentiated KN from the WLH, in form of private alleles that could be attributed to the variant geographical origin and distribution of these flocks coupled with their unique breeding histories.

The presence of population specific private alleles as observed here may act as important tool for identification of the respective population. The KN population by virtue of its propagation in a backyard and harsher-agro-climatic environment solely for its phenotypic attributes including black meat, black plumage, but not for productivity, could have retained higher diversity, especially with higher number of private alleles that remained undiluted due to the continued conservation-breeding at CARI. On the other hand, WLH registered less number of private alleles, which can be explained by the continuous selection programmes accompanying this breed since centuries (selection being synonymous with its Mediterranean origin), besides the unidirectional long-term selection (30 generations) for egg production at CARI. Similar to our findings on private alleles (16) in the WLH, fifteen private alleles emanating from ten STRs has been reported in a WLH flock by Davilla *et al*. (2009). Likened to our results for KN, Rajkumar *et al*. (2008) have observed a total of 103 population-specific alleles combined over Aseel and non-descript (Desi) populations while twenty five private alleles were recorded in the non-descript (Desi) chickens by Pirany *et al*. (2007).

Interestingly, many common alleles were revealed in both populations across most STRs, which largely reflected the conserved-regions of the domesticated-chicken genome shared by these breeds, following thousands of years since their evolution from a common ancestor: Red Jungle Fowl (Fumihito *et al.*, 1994).

The Nei's index refers to the unbiased heterozygosity existent in a population and lower Nei value was observed in our study for the WLH (0.37±0.25) than KN (0.60± 0.16) indicated presence of higher diversity in the KN. Higher Nei value as recorded for the KN is in accordance with higher estimates (0.67) reported by Pandey *et al*. (2005) for another Indian native breed: Ankleshwar. Comparable to the Nei's value realized for our WLH flock, Mahadeokumar *et al*. (2006) have reported a value of 0.313 in a sub-population of this WLH flock (IWH) which was separated 15 generations before and maintained as a closed flock under selection at a physically-different location of India (PDP, Hyderabad). The authors also reported comparable Nei's value of 0.358 for another contemporary WLH population: IWI which was introduced into India almost at the same time as the entry of IWH into CARI (Ayyagari *et al*. 1996). A quite evident reason for the inflated Nei value in KN was that: no-deliberate selection was ever practiced in this flock which allowed it to retain a higher genetic diversity, in contrast to the WLH flock.

The KN was recorded higher (1.06 ±0.33) than those of WLH (0.63 ± 0.45) for Shannon's information Index (I) which generally indicated species-diversity of a population. Obviously, with higher value of 'I', a higher diversity would be indicated which was the case with our KN flock as deviated from WLH possessing significantly lower value. Comparable 'I' values like that of our KN have been reported in various chicken breeds around the world which included, Isfahan native chickens (0.97) by Nasiri *et al*. (2007); non-descript Indian chicken populations

(1.67) by Pirany *et al*. (2007) and Ankleshwar chickens (1.4) by Pandey *et al*.(2005). However a moderate 'I' value (0.99) in respect of commercial WLH chickens has also been reported by Pirany *et al*. (2007) which was little higher than the value realized for our WLH flock.

The important F-statistic parameters: F_{IS}, F_{IT} and F_{ST} were recorded higher (0.292, 0.270 and 0.271) for WLH than the ones for KN (0.173, 0.129 and 0.164 respectively). The differences in F-statistic parameters: F_{IS}, F_{IT} and F_{ST} for the KN and WLH as registered in our study truly reflected the differential breeding-histories of these populations.

An inbreeding coefficient (F_{IS}) is actually a measure of the non-random association of alleles within an individual. Negative F_{IS} value was obtained for STRs: ADL0145, LEI 074 in KN population and for LEI074, ADL0034, and ADL0114 in WLH. The negative value of F_{IS} indicates the presence of excess heterozygotes in the population while positive value exhibits less heterozygotes. Accordingly, the higher value of F_{IS} means close relationship between the individuals. The KN population revealed a moderate F_{IS} summed over the examined loci, which was less than that of WLH. As such, pedigree data used to determine the inbreeding coefficient yielded an estimate of 0.19 (Table 1), which was close to the F_{IS} (0.173) estimated from STR analysis. However, inbreeding coefficient in respect of WLH employing the pedigree information (breeding data) provided an underestimate i.e. 0.133 compared to the F_{IS} (0.292) derived from the STR method. Ahlawat *et al*. (2007) observed an F_{IS} value of 0.127 for his KN samples and an even lower F_{IS} value for his WLH stocks (0.021) which were lower than our estimates.

However, on the issue of coherence of inbreeding coefficients calculated from the breeding data and F_{IS} values generated from STR based analysis in Japanese quails, Kim *et al*. (2007) have cited that microsatellite based F_{IS} estimation was not very effective as compared to its computation using actual population-parameters from pedigree. As such, Varying F_{IS} values for many local breeds has been reported in the literature including the estimates of 0.301 ±0.05 by Kaya *et al.* (2008); 0.020 by Chen *et al.* (2008), 0.184 by Liu *et al.* (2008); a mean F_{IS} of 0.056 by Davila *et al.* (2009) measured in multiple native chicken stocks and an average F_{IS} of 0.11 by Pirany *et al.* (2007) measured in various local chicken stocks.

The F_{IT} (Variation of individuals among total population) indicates the global deficit of heterozygotes across populations. The low F_{IT} value of 0.164 for our KN population is well supported by similar findings (0.18) by Chen *et al*. (2008) and the report of 0.164 as the mean F_{IT} realized from six different chicken populations (Pirany *et al*., 2007). An equivalent F_{IT} value (0.286) like that of our WLH flock has been reported by Davila *et al*. (2009).

The Wright's Fixation Index or Coefficient (Wright, 1978) of co-ancestry (F_{ST}) is an indicator of genetic diversity within a population. Lower F_{ST} value indicates higher relationships between the breeds and vice-versa. Accordingly, lower F_{ST} value yielded for KN would mean more diversity within the breed while higher value recorded for WLH would imply less diversity. Likewise, Davila *et al*. (2009) estimated a mean F_{ST} value of 0.244 while Pirany *et al*. (2007) observed an average value of 0.15. The higher values for all these three parameters in WLH than KN can be attributed to the effects of continuous selection in the former and random-mating practiced in the latter.

The cluster analysis which used to provide an assessment of current genetic inter-relationship among the individuals was uniquely distinct from each other, as derived for these two breeds in our study. The clustering pattern of these samples reflected through a combined dendrogram (Fig.3) exhibited distinctly-variant spread of samples and dispersal patterns for these two breeds. The Tree-topology not only revealed the diversified relationship between the samples for

respective breed, but also the distinct looseness in clustering of KN samples as against the relatively tight-clustering of WLH samples. When studied within the KN population alone, un-rooted dendrogram for KN revealed a total of four clusters that lead to eight sub-clusters, which formed a total of twelve distinct branches.

In contrast, WLH-samples accommodated themselves in three major clusters which tended to divide into five sub-clusters having not more than nine branches in all. The study of the faithfulness of clustering for the combined lot of samples (Fig.3) revealed that most of the WLH and KN samples bundled up within respective breeds, except for three outliers from WLH and two outliers from KN.

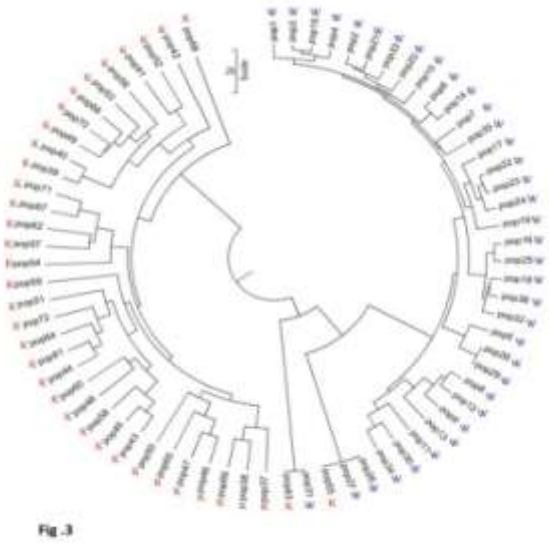


Fig.3

Such type of intermingling might be the result of less number of STRs used in the present study and minor mismatching in scoring of alleles. However, Comparative analysis of phylogenetic trees (from individual dendrograms not given here) depicted conclusively: how these breeds being different by geographical origin and possessing deviant breeding-histories are positioned at molecular level. Similar sort of Phylogenetic studies and interpretations from clustering patterns on origin and distribution of chicken populations, using microsatellite analyses like ours have been reported by many authors (Romanov and Weigend, 2001; Chain *et al*., 2008).

Summarized through the above population-parameters and diversity indices, the long-term selection programme practiced in the WLH appeared as the primary reason for reduced-polymorphism due to continuous loss of heterozygosity that accompanied reduced number of alleles across generations, while raising the purity-level of this stock.

6. CONCLUSIONS

Results of this study confirmed the efficiency of microsatellite markers and computational analytic tools for the evaluation of genetic variations within and between such geographically-distinct chicken populations. It was concluded that a panel of twelve STRs was sufficient for delineating genetic diversity between these two breeds. But, for enabling a full-proof molecular differentiation between randomly-drawn samples from these breeds, a larger panel of STRs would be necessary.

7. ACKNOWLEDGEMENT

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Table 1. Diversity indices and population parameters derived from Molecular analyses for KN and WLH breeds

Locus	Pop	PIC	na*	ne*	I*	F_{IS}	F_{ST}	F_{IT}	Nei**	He	Ho	Av Het.	Private Alleles (Frequency)	Fixed Alleles (Frequency)
ADL0145	KN	0.50	3	2.308	0.953	-0.113	0.058	-0.120	.567	0.575	0 .639	0.3194	129 (0.29),144 (0.58)	115 (0.15)
	WLH	0.38	2	2.000	0.693	0.014	0.166	-0.137	0.500	0.507	0.500	0.2500	146 (0.50)	115 (0.50)
ADL0185	KN	0.55	3	2.664	1.028	0.169	0.167	0.217	0.625	0.633	0.528	0.2639	144(0.43),161 (0.40)	132 (0.17)
	WLH	0.45	3	2.000	0.877	0.208	0.302	0.225	0.520	0.524	0.417	0.2083	140 (0.64),150 (0.11)	132 (0.25)
ADL0102	KN	0.77	6	5.041	1.651	0.088	0.122	0.067	0.802	0.813	0.743	0.3611	101(0.16),112(0.23),125(0.17)	98 (0.21) ,118(0.21),109 (0.01)
	WLH	0.66	5	3.475	1.391	0.117	0.185	0.160	0.712	0.722	0.639	0.3194	105 (0.07), 135 (0.11)	98(0.35), 18(0.38),109(0.10)
ADL0278	KN	0.29	2	1.545	0.538	0.042	0.024	-0.034	0.353	0.358	0.343	0.1667	109 (0.23)	118 (0.77)
	WLH	0.29	2	1.528	0.531	1.00	0.702	1.000	0.346	0.351	0.000	0.0000	120 (0.22)	118 (0.78)
ADL0202	KN	0.71	5	4.050	1.477	0.312	0.145	0.152	0.753	0.764	0.528	0.2639	238(0.1), 243(0.3) 250(0.3),257 (0.7)	248 (0.25)
	WLH	0.00	1	1.000	0.000	NA	0.000	NA	0.000	0.000	0.000	0.0000	NIL	248 (1.0)
MCW0005	KN	0.44	3	1.971	0.853	0.260	0.092	0.283	0.493	0.500	0.371	0.1806	218(0.7),221(0.13),238 (0.2)	NIL
	WLH	0.64	4	3.319	1.272	0.297	0.322	0.274	0.699	0.709	0.500	0.2500	234(0.08),243(0.4),251(0.3),276(0.3)	NIL
Lei 074	KN	0.57	4	2.762	1.159	-0.240	0.068	-0.278	0.638	0.647	0.800	0.3889	315 (0.49),330(0.33)	306(0.11),325(0.07)
	WLH	0.10	2	1.117	0.215	0.045	0.145	-0.086	0.105	0.106	0.111	0.0556	NIL	306 (0.9),325 (0.1)
ADL0034	KN	0.25	3	1.369	0.528	0.087	0.111	0.081	0.270	0.274	0.250	0.1250	112(0.06),123 (0.84)	152 (0.09)
	WLH	0.26	2	1.456	0.493	0.228	0.132	-0.227	0.313	0.318	0.389	0.1944	127(0.81)	152(0.19)
MCW0217	KN	0.58	4	2.839	1.138	0.264	0.247	0.331	0.648	0.657	0.486	0.2361	181(0.46),187(0.03)	153(0.46),157 (0.19)
	WLH	0.26	2	1.456	0.493	1.000	0.351	1.000	0.313	0.318	0.000	0.0000	NIL	153(0.8),157 (0.19)
ADLO176	KN	0.66	4	3.557	1.312	0.337	0.139	0.359	0.719	0.729	0.486	0.2361	183(0.10),186(0.31),204(0.27)	199(0.31)
	WLH	0.58	3	2.906	1.082	0.502	0.238	0.487	0.656	0.665	0.333	0.1667	189(0.4),193(0.25)	199(0.4)
ROSO 302	KN	0.55	3	2.624	1.027	0.161	0.191	0.183	0.619	0.628	0.528	0.2639	104(0.5),107(0.19),109(0.31)	NIL
	WLH	0.00	1	1.000	0.000	NA	0.000	NA	0.000	0.000	0.000	0.0000	111 (1.0)	NIL
ADL0114	KN	0.58	3	2.916	1.084	0.545	0.106	0.562	0.657	0.666	0.306	0.1528	175(0.4)	162 (0.26)
	WLH	0.25	3	1.369	0.528	-0.119	0.109	-0.129	0.270	0.274	0.306	0.1528	178 (0.10) ,181(0.06)	162(0.85)
Overall	KN	0.54	3.58 ±1.08	2.80 ±1.03	1.06 ±0.33	0.173	0.129	0.164	0.60 ±0.16	0.60 ±0.16	0.50 ±0.17	0.25 ±0.82		
	WLH	0.32	2.5 ±1.17	1.89 ±0.88	0.63 ±0.45	0.292	0.270	0.271	0.37 ±0.25	0.37 ±0.25	0.27 ±0.23	0.133 ±0.11		

na = Observed number of alleles; * ne = Effective number of alleles ; # I = Shannon's Information inde*x; Wright's fixation index (F_{IS}) is a measure of heterozygote deficiency or excess ; ** Nei's expected heterozygosity. (NA indicates: no estimates were computable due to isomorphism at these loci)*