

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Variability of Microsatellites and their Association with Egg Production Traits in Chicken

R.N. Chatterjee, R.P. Sharma, A. Mishra, M. Dange and T.K. Bhattacharya
Project Directorate on Poultry, Rajendranagar, Hyderabad-500030, Andhra Pradesh, India

Abstract: The present study was conducted on six crossbred chicken populations of White Leghorn to estimate variability of microsatellites and their association with egg production traits. Five microsatellite markers located on chromosome 1, 2, 5 and 30 were explored and the association study was performed employing least square-maximum likelihood method. All the microsatellites were found to be polymorphic showing three to six alleles in the population. Genotype and allelic frequency was estimated showing a large variability in different microsatellites. The association study of microsatellite variability with egg production traits showed that only ADL023 microsatellite was significantly associated with egg production upto 64 and 72 weeks and egg weight at 28 weeks of age. Genotype 11, 12, 13 and 23 produced more number of eggs at 64 and 72 weeks of age than the genotype 22. Egg weight was higher in genotype 12, 13 and 23 and lower with genotype 11 and 22.

Key words: Association, chicken, egg, microsatellite

Introduction

Egg production traits are quantitative in nature with a continuum between high and low-performing birds. The regions of the genome that control such traits are termed as quantitative trait loci (QTL) and the markers flanking such QTL can be used in marker assisted selection to introduce or retain beneficial QTL allele. However, markers have to be very closely linked to the causative mutation in the trait gene if they are to remain associated with specific QTL alleles through several generations of selection and therefore, be useful in practical breeding programmes. To detect such QTLs, one of the approaches is genome scan approach under which microsatellites can be studied as they are highly polymorphic repetitive DNA sequences and randomly distributed throughout the genome displaying high levels of variation and consequently, are ideal for deciphering genetic variability. Microsatellite markers, by virtue of their codominancy and multiple-allelism proved to be efficient in genetic diversity studies, pedigree evaluation and genetic mapping as compared to other molecular markers like RAPD, RFLP and ISSRs (Ahlawat *et al.*, 2004; Chatterjee *et al.*, 2007 and Nagaraju *et al.*, 2001). Chromosome 1 localizes growth related genes like IGF1, lactate dehydrogenase B, TPT1, myosin heavy chain, egg number and egg weight etc. Chromosome 2 bears egg number, egg weight along with other QTLs. Chromosome 5 carries IGF2, HAS2 (carbohydrate metabolism), LOC421012 (metabolic enzyme), EGF-like2 for cartilage development, egg number and egg weight etc. Chromosome 5 also bears LOC395381 (ovomucin gene) related to reproductive function in chicken (Abasht *et al.*, 2006a). Abasht *et al.* (2006b) reviewed on QTLs in chicken and observed that

some QTLs for egg number and egg weight are located on chromosome number 5, besides chromosome number 1 and 2. Thus, these chromosomes have become the hotspot zones for studying QTLs for production traits in poultry birds. In general, microsatellite provides maximum polymorphic information content of the genome and thus, the microsatellites located on these chromosomes have been considered to explore the association of their variability with egg production traits.

Thus the present study was conducted to observe the variability of microsatellite markers and their association with the egg production traits in different crossbred chicken populations.

Materials and Methods

Genetic stock: The present study was conducted on 6 crossbred layer chickens produced by utilizing 3 pure lines of White Leghorn populations namely IWH, IWI and IWK maintained at Project Directorate on Poultry farm, Hyderabad, A.P., India. The genetic groups studied were IWH X IWI, IWI X IWH, IWK X IWH, IWH X IWK, IWI X IWK and IWK X IWI. The IWH and IWI were selected for egg number and egg weight for over 10 generations and IWK for feed efficiency and latter on for egg mass for over 10 generations. The study was carried out on 15 birds of each crossbred population and birds were unrelated and selected randomly for the present study.

Sample and DNA: Approximately 0.5 ml blood was collected from each bird and genomic DNA was isolated following phenol-chloroform extraction method (Sambrook and Russel, 2001). The quantity and quality of DNA was evaluated on spectrophotometer and through 0.8% agarose gel electrophoresis.

Chatterjee *et al.*: Variability of Microsatellites and their Association with Egg Production Traits in Chicken

Table 1: Microsatellites, their amplification condition and alleles resolved in crossbred chicken population

Sl. No.	Marker name	Primer Sequence (5'-3')	Chromosomal location	Annealing temperature (°C)	MgCl ₂ concentration (mM)	Alleles (bp)
1.	MCW007	AGCAAAGAAGTGTCTCTGTTTCAT ACCGTGCAAACCTGGAAGGGTCTCA	1	62	0.75	292, 298, 320
2.	ADL020	GCACTCAAAGAAACAAT TAGATAAAAATCCTTCCCTT	1	55	3.0	97, 107, 112
3.	ADL023	CTTCTATCCTGGGCTTCTGA CCTGGCTGTGTATGTGTGC	5	61	1.5	176, 184, 204
4.	ADL102	TTCCACCTTCTTTTTTATT GCTCCACTCCCTCTAACCC	30	48	1.5	92, 108, 118
5.	ADL176	TTGTGGATTCTGGTGGTAGC TTCTCCCGTAACACTCGTCA	2	54	1.5	138, 150, 160, 168, 176, 188

Table 2: Microsatellite polymorphism and their frequencies in crossbred chicken

Micro satellite locus	Genotypes													
	11	12	13	14	16	22	23	25	26	33	34	36	44	56
MCW007	0.04	0.48	0.04	0.04	0.03	0.01		0.04	0.01	0.19	0.03	0.03	0.05	0.01
ADL020	0.15	0.33	0.16	-	-	0.16	0.11	-	-	0.09	-	-	-	-
ADL023	0.04	0.52	0.39	-	-	0.01	0.04	-	-	-	-	-	-	-
ADL102	0.15	0.40	0.39	-	-	0.01	-	-	-	0.05	-	-	-	-
ADL176	0.23	0.32	0.28	-	-	0.04	0.06	-	-	0.06	-	-	-	-
	----- Alleles -----													
		1		2			3		4		5		6	
MCW007		0.54		0.21			0.25		-		-		-	
ADL020		0.53		0.23			0.24		-		-		-	
ADL023		0.49		0.29			0.21		-		-		-	
ADL102		0.39		0.38			0.23		-		-		-	
ADL176		0.33		0.28			0.23		0.09		0.03		0.04	

Microsatellites: Five microsatellites namely, MCW007, ADL020, ADL023, ADL102 and ADL176 which were located on chromosome no. 1, 2, 5 and 30 were considered for the present study as these chromosomes harbor genes controlling growth, reproduction and disease resistance traits. Chromosome1 presents MCW007 and ADL020 while chromosome2 carries ADL176 microsatellite. Chromosome5 bears ADL023 and chromosome30 presents microsatellite ADL102.

Polymerase chain reaction (PCR): PCR was performed in 25 µl reaction mixture containing 100-200ng DNA template, 20 pM of each primer, 200 µM each dNTP, 1U Taq DNA polymerase and optimised quantity of MgCl₂ (Table 1). The optimum annealing temperatures which gave the best amplification has been presented in Table1.

Poly acrylamide gel electrophoresis: Amplified products were electrophoresed at 4°C on 8% non-denaturing polyacrylamide gel containing acrylamide and bis-acrylamide in the ratio of 29:1. The gel was run at 250V for 4 hrs in 1X TBE and stained with 0.1% silver nitrate following the standard protocol (Bhattacharya *et al.*, 2007). The gel was visualized and documented under white light of gel documentation system.

Traits: The daily egg production was recorded upto 72

weeks of age and consequently, egg production upto 28, 40, 52, 64 and 72 weeks of age were calculated. Egg weights were also measured at 28, 40, 52 and 64 weeks of age. All these traits were considered for the present study.

Genotyping: Genotype of every animal was determined. Genotyping involved the recording of the homozygous or heterozygous state of the animal, as well as the size of the respective alleles. The size of the allele was estimated by comparing with standard ladder DNA marker using Quantity One 4.2.3 software (Biorad Laboratories, USA). Ultimately, the frequencies of different alleles were estimated in different breed groups following gene-counting method.

Association: The association studies of genotypes with egg production and weight traits were carried out employing least squares-maximum likelihood method (Harvey, 1991).

Results

Genotypes: All the microsatellites were found to be polymorphic with the presence of three to six alleles in crossbred layer chicken populations (Table 1). Out of all the microsatellites, ADL176 was observed to be the highest polymorphic marker showing six alleles distributing over the lines.

The Genotype frequencies for MCW007, ADL020,

Chatterjee *et al.*: Variability of Microsatellites and their Association with Egg Production Traits in Chicken

Table 3: Least square means of various egg production traits under different genotypic groups of crossbred chicken.

Micro satellite	Ew28	Ew40	Ew 52	Ew 64	Ep28	Ep40	Ep52	Ep64	Ep72
Genotype	(gm)	(gm)	(gm)	(gm)					
MCW007									
11	46.7±0.39	46.7±0.16	49.1±0.18	54.2±0.19	37±2	103±7	157±10	208±13	235±16
12	48.9±0.36	48.0±0.15	50.7±0.17	54.2±0.17	33±2	97±6	156±9	208±12	241±14
13	52.2±0.33	49.6±0.14	51.9±0.15	54.2±0.16	37±2	102±5	163±8	215±11	246±13
22	42.6±0.85	50.1±0.36	51.5±0.40	50.6±0.41	39±6	104±15	163±22	204±28	226±35
33	43.2±0.61	46.6±0.26	49.6±0.28	52.0±0.29	38±4	103±11	151±16	199±20	231±25
ADL020									
11	47.3±0.38	46.0±0.16	50.8±0.18	54.8±0.18	39±2	106±7	165±10	215±13	243±15
12	46.1±0.36	49.8±0.15	50.6±0.17	54.5±0.17	36±2	101±6	156±9	209±12	240±14
13	45.1±0.38	48.6±0.16	49.5±0.18	53.5±0.18	39±2	104±7	160±10	210±13	240±15
22	46.4±0.69	46.5±0.29	48.5±0.32	50.1±0.33	39±5	98±12	155±18	203±23	238±28
23	48.6±0.57	53.1±0.24	53.5±0.27	52.4±0.28	35±4	99±10	156±15	204±19	226±23
33	46.8±0.54	45.3±0.23	50.3±0.25	52.9±0.26	34±4	102±9	157±14	200±18	227±22
ADL023									
11	32.9±0.60 ^b	49.5±0.26	47.9±0.28	54.3±0.29	31±4	105±10	159±16	213±20 ^a	240±24 ^a
12	52.2±0.27 ^a	44.8±0.11	52.3±0.13	53.9±0.13	41±2	108±4	167±7	220±9 ^a	249±1 ^a
13	52.0±0.28 ^a	44.3±0.12	50.5±0.13	53.8±0.13	40±2	108±5	169±7	221±9 ^a	253±11 ^a
22	39.8±0.96 ^b	48.6±0.41	44.6±0.45	47.4±0.46	34±7	92±17	142±25	165±32 ^b	194±39 ^b
23	56.6±0.62 ^a	49.9±0.26	57.4±0.29	55.8±0.30	37±4 ^b	96±11	153±16	214±21 ^a	243±25 ^a
ADL102									
11	47.5±0.48	46.1±0.20	47.3±22	51.5±0.23	37±3	101±8	162±12	210±16	240±19
12	44.9±0.43	47.5±0.18	49.1±0.20	52.4±0.21	37±3	105±7	164±11	212±14	242±17
13	48.3±0.43	47.6±0.18	50.6±0.20	53.5±0.21	36±3	103±7	161±11	207±14	238±18
22	44.9±0.44	46.3±0.19	48.5±0.21	52.0±0.21	37±3	101±8	154±12	199±15	225±18
23	49.8±0.43	51.0±0.18	54.9±0.20	53.1±0.21	36±3	103±7	160±11	212±14	239±17
33	44.8±0.50	50.8±0.21	52.9±0.23	55.7±0.24	36±3	98±9	148±13	200±16	232±20
ADL176									
11	49.7±0.62	51.0±0.26	54.4±0.29	54.6±0.30	41±4	114±11	171±16	223±20	259±25
12	46.8±0.32	48.4±0.13	49.2±0.15	53.8±0.15	35±2	101±5	156±8	206±10	233±13
13	51.2±0.57	44.5±0.24	49.7±0.27	49.6±0.27	32±4	101±10	160±15	206±19	228±23
14	47.9±0.56	48.4±0.24	48.8±0.26	52.2±0.27	36±4	110±10	169±15	219±19	244±23
16	32.7±0.79	48.4±0.34	52.3±0.37	54.1±0.38	41±5	108±14	179±21	222±26	245±32
22	43.5±0.97	44.9±0.41	44.9±0.45	54.2±0.47	30±7	101±17	158±26	201±32	241±40
25	43.4± 64	50.0±0.27	51.2±0.30	50.9±0.31	32±4	94±11	140±17	197±21	233±26
26	47.7±0.97	45.2±0.41	51.2±0.45	54.0±0.47	42±7	93±17	154±26	197±32	224±40
33	47.7±0.42	48.8±0.18	51.3±0.20	54.6±0.20	38±3	105±7	161±11	212±14	237±17
34	49.6±0.69	47.1±0.30	48.3±0.32	54.3±0.33	32±5	98±12	145±18	199±23	234±28
36	55.6±0.71	55.0±0.30	58.2±0.33	50.5±0.34	30±5	92±12	144±19	196±24	225±29
44	49.0±0.54	51.4±0.23	51.5±0.25	50.7±0.26	33±4	96±9	148±14	192±18	216±22
56	42.5±0.84	43.9±0.36	45.9±0.39	55.7±0.41	54±6	110±15	171±22	218±28	247±34

EW28, EW40, EW52 and EW64 = Egg weight at 28, 40, 52 and 64 weeks of age; EP28, EP40, EP52, EP64 and EP72 = Egg production upto 28, 40, 52, 64 and 72 weeks of age. Different superscripts indicate significance at P<0.05.

ADL023, ADL102 and ADL176 microsatellite were varied from 0.01 to 0.48, 0.09 to 0.33, 0.01 to 0.52, 0.01 to 0.40 and 0.04 to 0.32, respectively whereas the allele frequencies were found to be in the range of 0.21 to 0.54 for MCW007, 0.23 to 0.53 for ADL020, 0.21 to 0.49 for ADL023, 0.23 to 0.39 for ADL102 and 0.23 to 0.33 for ADL176 marker (Table 2).

Association with egg production traits: The association study of microsatellite variability with egg production traits showed that only ADL023 microsatellite was found to be significantly (P<0.05) associated with egg production upto 64 and 72 weeks. This microsatellite has also been significantly associated (P<0.05) with egg weight at 28 weeks of age (Table 3).

Discussion

Genotypes: All the microsatellites were found to be polymorphic with the presence of three to six alleles in crossbred layer chicken populations (Table 1). Out of all the microsatellites, ADL176 was observed to be the highest polymorphic marker showing six alleles distributing over the lines.

The Genotype frequencies for MCW007, ADL020, ADL023, ADL102 and ADL176 microsatellite were varied from 0.01 to 0.48, 0.09 to 0.33, 0.01 to 0.52, 0.01 to 0.40 and 0.04 to 0.32, respectively whereas the allele frequencies were found to be in the range of 0.21 to 0.54 for MCW007, 0.23 to 0.53 for ADL020, 0.21 to 0.49 for ADL023, 0.23 to 0.39 for ADL102 and 0.03 to 0.33 for ADL176 marker (Table 2).

Our study revealed that the genotypic proportion were distributed from low to moderate whereas the allelic frequencies were ranging from 21 to 54%. In ADL176, some alleles were rare with the existence of only 3 to 4% in the population. These alleles may be in the path of extinction from the population and are mostly present in heterozygotic form. Osman *et al.* (2004) used microsatellites for studying genetic variability in the Oh-Shamo and its related chicken breeds emphasizing the potential of large genetic variability in birds.

Association with egg production traits: The association study of microsatellite variability with egg production traits showed that only ADL023 microsatellite was found to be significantly ($P < 0.05$) associated with egg production upto 64 and 72 weeks. This microsatellite has also been significantly associated ($P < 0.05$) with egg weight at 28 weeks of age (Table 3). It is known that ADL023 microsatellite is located on chromosome 5 which harbors genes regulating fat synthesis (Abasht *et al.*, 2006a) and ovomucin for controlling reproduction in chicken. It is obvious that fat synthesis mechanism is one of the important criteria for egg production as egg yolk is highly rich in fat. Consequently lipid bio-synthesis path way has become very much important for egg production and egg weight. One to one correspondence in the form of significance between the microsatellite with egg production and weight has possibly been the informative indicator for elucidating QTL and microsatellite relationship. The degree of relationship between microsatellite and production traits has gained a momentum for exploring markers with respect to egg traits in chicken lines. There is a genetical fact that if microsatellite is linked with certain chromosomal segment regulating specific phenotypes, it will determine the significant association between microsatellite and those phenotypes. Genotypes 11, 12, 13 and 23 produced more number of eggs than genotype 22. The egg laying performance of 11, 12, 13 and 23 genotypes did not differ significantly among each other and their yield were varied from 213 to 221 upto 64 weeks and 240 to 253 upto 72 weeks of age. The genotype 22 produced poor performance by about 25% lower than genotype 13 which was the highest producing genotype. There is a trend that wherever there were the allele 3 or allele 1, the respective genotypes were producing better. Thus, we suggest that allele 1 or 3 possibly better for egg production in chicken. Further, egg weight was better in case of genotype 12, 13 and 23 and the lower egg weight was observed in birds with genotype 11 and 22 for microsatellite ADL023. Overall, the presence of allele 3 was correlated with better egg production as well as egg weight. Tuiskula - Haavisto *et al.* (1998) exploited microsatellite variation for elucidation of markers for egg quality traits in chicken. Abasht *et al.* (2006b) reviewed on QTLs in chicken and

observed that some QTLs for egg number and egg weight are located on chromosome number 5.

In conclusion we can say that the allele 3 of microsatellite ADL023 could be exploited for selection of birds for better egg production and early egg weight.

References

- Abasht, B., F. Pitel, S. Lagarrigue, E.L. Duval, P.L. Roy, O. Demeure, F. Vignoles, J. Simon, L. Cogburn, S. Aggrey, A. Vignal and M. Douaire, 2006a. Fatness QTL on chicken chromosome 5 and interaction with sex. *Genet., Selec. Evol.*, 38: 297-311.
- Abasht, B., J.C.M. Dekkers and S.J. Lamont, 2006b. Review of Quantitative trait loci identified in the chicken. *Poult. Sci.*, 85: 2079-2096.
- Ahlawat, S.P.S., J. Sunder, A. Kundu, R.N. Chatterjee, R.B. Rai, B. Kumar, S. Senani, S.K. Saha and S.P. Yadav, 2004. Use of RAPD-PCR for genetic analysis of Nicobari fowl of Andamans. *Br. Poult. Sci.*, 45: 1-7.
- Bhattacharya, T.K., S. Rani, S.K. Maiti, S. Dayal, P. Kumar and A. Sharma, 2007. Polymorphism of ZuBeCa3 microsatellite and its association with mammary tumor in dog. *Inter. J. Immunogenet.* (In press).
- Chatterjee, R.N., R.P. Sharma, B.L.N. Reddy, M. Niranjana, Shivaprasad and S.K. Mishra, 2007. Genetic analysis of highly inbred chicken using RAPD-PCR and immunocompetence. *Inter. J. Poult. Sci.*, 6: 967-972.
- Harvey, W.R., 1991. User's guide for LSMLMW, mixed model least squares and maximum likelihood computer programme. Ohio State University (Mimeograph).
- Nagaraju, J., K.D. Reddy, G.M. Nagaraja and B.N. Sethuraman, 2001. Comparison of multilocus RFLPs and PCR-based marker systems for genetic analysis of the silkworm *Bombyx mori*. *Hered.*, 86: 588-597.
- Osman, S.A., M. Sekino, M. Nishibori, Y. Kawamoto, K. Kinoshita, Y. Yamamoto and M. Tsudzuki, 2004. Genetic variability and relationships of Japanese native chickens assessed by means of microsatellite profiling approach-focussing on the Oh-Shamo (Japanese large game) and its related breeds. *J. Poult. Sci.*, 41: 94-109.
- Sambrook, J. and D.W. Russel, 2001. Molecular Cloning: A laboratory manual. 3rd (Edn). Vol. 1. Cold Spring Harbor Laboratory press. New York, USA.
- Tuiskula-Haavisto, M., M. Honkatukia, J. Vilkki, D.J. Koning, N. Schulman and A. Maki-Tanila, 1998. Use of microsatellite markers for localizing genes affecting egg quality in chicken. *Proceedings of 6th World Congress on Genetics and Applied to Livestock Production. Armidale, Australia, Vol. 23-28.*