Association of Microsatellites with Growth and Immunocompetence Traits in Crossbred Layer Chicken

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The present investigation was carried out on six crossbred chicken populations to estimate variability of microsatellites and their association with growth and other traits. Five microsatellite markers located on chromosome 1, 2, 5 and 10 were screened and association study was performed following general linear model technique. All the microsatellites were polymorphic showing three to six alleles. The polymorphic information content (PIC) of the markers was more than 0.536. Genotype and allelic frequency was estimated showing a large variability from microsatellite to microsatellite. The genotypes of *MCW007* microsatellite were found to be significantly ($P < 0.05$) associated with body weight at day old, 8th, 12th, 20th, 28th and 40th week of age. A significant association between *ADL020* microsatellite and body weight at 8th, 12th and 40th week of age was estimated at $P < 0.05$ in different crossbred chicken populations. *ADL176* genotypes were observed to be significantly associated with body weight at 40 weeks of age. *MCW007, ADL020, ADL023* and *ADL176* microsatellites were found to be significantly correlated with age at sexual maturity whereas humoral immune response to sheep RBC were observed to be non-significantly associated with the microsatellites.

Key words: chicken, growth, immunocompetence, microsatellite

Introduction

The poultry farming has been the backbone of rural poor people providing their livelihood in the form of poultry meat and egg. Poultry egg industry is one of the most emerging field of poultry farming producing very cheap source of animal proteins. Physiologically if poultry birds are healthy, they would be better in production as well as reproduction. It is a fact that age at sexual maturity depends on the body weight and if birds attain a particular body weight at early age, the age at sexual maturity will be lower, ultimately they will produce at an early age of life. Thus, birds will produce more eggs at its productive life. General immunocompetence which is assessed by humoral and cell mediated immunity and phagocytic index is important in poultry because birds with better immunocompetence will have better survivability and more resistant to disease. Humoral response to sheep RBC (SRBC) titre is one facet of judging the general immune competence.

Growth traits are quantitative in nature with a continuum between high and low-performing birds. Thus, the regions of the genome that control such traits are termed as quantitative trait loci (QTL). QTL can be defined as the marker interval that co-segregates with variation in the traits of interest. The markers including such intervals 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 approach is genome scan under which microsatellites can be studied as they are highly polymorphic repetitive DNA sequences and are randomly distributed through out the genome displaying high levels of variation and consequently, are ideal for deciphering genetic variability. They are having high mutation rates (1–4 per generation). Earlier studies reported that QTLs for body weight are located in chromosome 1 and 5 (Abasht *et al.*, 2006). Van Kaam *et al.* (1998, 1999) found QTL for body weight at the age of 48 day on chromosome 1 whereas by Ikeobi *et al.* (2002) reported a QTL for abdominal fatness in the same chromosome. Jennen *et al.* (2004) revealed significant association of microsatellites with body weight at 10 weeks of age and abdominal fat percentage in chicken. Hence, microsatellites located on such chromosomes have certain added value to explore genetic markers associated with

growth QTLs in poultry birds. The present investigation was undertaken to explore variability of microsatellites and their association with growth traits in various cross-bred chickens.

Materials and Methods

Experimental Birds

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, Andhra Pradesh, 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 12–15 birds of each crossbred population, and birds were unrelated, and selected randomly for the present study.

Sample Collection

Approximately 0.5 mL venous blood was collected from each animal in eppendorf tubes containing $100\,\mu$L 0.5 M EDTA as anticoagulant and brought to laboratory under low temperature. Finally, all the samples were kept at -20℃ till DNA was isolated.

Genomic DNA Extraction

High molecular weight genomic DNA was prepared using phenol-chloroform extraction method (Sambrook and Russell, 2001) with minor modifications. The quantity and quality of DNA was evaluated on spectrophotometer and through 0.8% agarose gel electrophoresis.

Selection of Microsatellite Markers

A total of five microsatellites, which were located on chromosome 1, 2, 5 and 10 were selected for the present study. These chromosomes were considered because they are probably the one of the hot spots of QTLs in relation to growth, reproduction and certain immunity traits in chicken.

PCR

A panel of 5 microsatellites were used for the present study and they have been presented in Table 1. PCR was performed in $25\,\mu$L reaction mixture containing 100–200 ng DNA template, $20\,\mu$M of each primer, $100\,\mu$M each dNTP, 1 U *Taq* DNA polymerase, optimised quantity of MgCl$_2$ and 10 ×PCR assay buffer (Bhattacharya *et al.,* 2007). The optimum annealing temperatures which gave the best amplification was mentioned in Table 1.

Electrophoresis

PCR products were electrophoresed at 4℃ in a 8% non-denaturing polyacrylamide gel containing acrylamide and bis-acralymide in the ratio of 29: 1. The gel was run at 250V for 4 h in 1X TBE and stained with 0.1% silver nitrate following the improved protocol of Bassam *et al.* (1991) and Bhattacharya *et al.* (2007).

Documentation and Estimation

The gel was visualized and documented under white light of gel documentation system.

Measurements of Traits

The birds were measured individually for body weight at different ages (at day old, 8 weeks, 12 weeks, 20 weeks, 28 weeks and 40 weeks of age) and age at sexual maturity (ASM). Before measurement of Age at sexual maturity, the birds were housed in individual cages. The age in days, at the time of laying its first egg, was taken as the measure of ASM in individual pullets. The body weights were measured with a balance at different ages as mentioned.

Estimation of Humoral Response

Humoral response was estimated in the form of titre against sheep RBC. Each bird was received an intravenous injection of 0.1 mL of 0.5% suspension of packed SRBC in normal saline at 6 weeks of age. Five days later, the blood was collected from wing vein of each bird in the individual test tubes. Sera were collected after two hours of incubation at room temperature and kept at -40℃. The total antibody titre was determined by haemagglutination test performed in microtitre plates. From individual serum sample two fold serial dilutions was carried out in normal saline solution and equal amount of 2% SRBC suspension was added in each well. The serum was not added in the control well. The plates were incubated at 37℃ for about 1 h. The reciprocal of highest dilution showing 50% agglutinations was expressed as titre (n). The titer was transformed into $\log_2^{(n+1)}$ for further analysis.

Statistical Analysis

Genotyping

Genotype of every animal was recorded manually from the gel. Genotyping involved the recording of the homo-

Table 1. **Amplification condition and allele size of microsatellite markers in chicken population**

Sl No.	Microsatellite	Chromosomal No.	Annealing temperature (℃)	MgCl$_2$ concentration (mM)	No. of alleles	Alleles (bp)
1	*MCW007*	1	62	0.75	3	292, 298 & 320
2	*ADL020*	1	55	3.0	3	97, 107 & 112
3	*ADL023*	5	61	1.5	3	176, 184 & 204
4	*ADL102*	10	48	1.5	3	92, 108 & 118
5	*ADL176*	2	54	1.5	6	138, 150, 160, 168, 176 & 188

zygous or heterozygous state of the animal, as well as the size of the respective alleles. The size of alleles was estimated by comparing standard ladder marker with Quantity One 4.2.3 software (Biorad Laboratories, USA). The frequencies of different alleles were estimated in different breeds following gene-counting method. The polymorphic information content (PIC) and heterozygosity were calculated following the standard formula.

Association

The association studies of genotypes with different growth traits, age at sexual maturity and humoral response were conducted following general linear model technique (LSML 91) (Harvey, 1991).

Results

Microsatellite Finger Printing

Total five microsatellite markers namely, *MCW007, ADL020, ADL023, ADL102* and *ADL176* were screened in the six crossbred layer chicken populations. *MCW007* microsatellite showed the presence of five genotypes namely, 11, 12, 13, 22 and 33 across all the population and consequently, evolved three alleles such as 1, 2 and 3. The allele size was varied from 292 to 320 bp. *ADL020* microsatellite indicated polymorphism having the presence of

six genotypes and three alleles namely, 1, 2 and 3. The allele sizes were ranged from 97 to 112 bp. Likewise, *ADL 023* and *ADL102* were observed to be the polymorphic capitalizing the presence of five genotypes and three alleles each in the crossbred population. The allele sizes in these two microsatellites were varied from 176 to 204 bp for *ADL023* and 92 to 118 bp for *ADL102* (Table 1). In case of *ADL176* marker, thirteen genotypes were found and consequently, six alleles ranging from 138 to 188 bp were resolved in the study.

Frequency Distribution

The frequencies of five informative marker loci have been presented in Table 2. The Genotype frequencies for *MCW007, ADL020, ADL023, ADL102* and *ADL176* microsatellite were varied from 0.01 to 0.40, 0.04 to 0.32, 0.01 to 0.52, 0.09 to 0.33 and 0.01 to 0.48, respectively. 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 3).

Marker Informativeness

The marker informativeness were calculated in the form of number of alleles, PIC and heterozygosity (Table 4). The PIC values for the markers were varied from 0.536 to

Table 2. **Genotype frequencies of microsatellites in crossbred chicken**

Microsatellite	Genotypes													
	11	12	13	14	16	22	23	25	26	33	34	36	44	56
MCW007	0.23	0.32	0.28	—	—	0.04	0.06	—	—	0.06	—	—	—	—
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	0.04	0.48	0.04	0.04	0.03	0.01	—	0.04	0.01	0.19	0.03	0.03	0.05	0.01

Table 3. **Frequencies of microsatellite alleles in chicken**

Microsatellite	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

Table 4. **Informativeness of microsatellite markers in chicken**

Sl No.	Microsatellite	Number of alleles	PIC value	Expected heterozygosity	Observed heterozygosity
1	*MCW007*	3	0.55	0.61	0.71
2	*ADL020*	3	0.54	0.61	0.60
3	*ADL023*	3	0.58	0.64	0.95
4	*ADL102*	3	0.58	0.65	0.79
5	*ADL176*	6	0.72	0.75	0.66

0.719. The heterozygosity of these microsatellites were found to be in the range of 0.610 to 0.756.

Association with Growth Traits

All the body weight parameters reflecting growth have been delineated in Table 5 showing variabilities with respect to different genotypic groups. The higher magnitude of standard deviation of each trait has been depicted with the corresponding standard error values of the trait. Least square analysis incorporating different factors such as breed group and genotypes was employed to estimate the association of genotypes with growth traits. The genotypes of *MCW007* microsatellite were found to be significantly ($P < 0.05$) associated with body weight at day old, 8th, 12th, 20th, 28th and 40th week of age (Table 5). Birds with genotype 11, 12, 13 and 22 were having higher day old body weight than genotype 33. But, as age progressed, the contribution of genotype towards body weight was changed. However, genotype 11 was having relatively higher body weight at all the age groups. Besides, genotypes having allele 1 was also showing higher body weight than other genotypes. A significant association between *ADL020* microsatellite and body weight at 8th, 12th and 40th week of age was estimated at $P < 0.05$ in crossbred chicken population. In this microsatellite, genotype 11 was showing relatively higher body weight at 8th, 12th as well as 40 weeks of age. But, birds having at least one allele 2 showed relatively lower body weight. Finally, it may be concluded that birds with allele 1 should be preferred in the population and would produce better body weight. *ADL176* genotypes were observed to be

Table 5. **Least square means along with standard errors of various economic traits under different genotypic groups of crossbred chicken**

Geno-type	D wt (g)	8 wt (g)	12 wt (g)	20 wt (g)	28 wt (g)	40 wt (g)	Asm (days)	Titre
MCW007								
11	34.4±0.17[a]	425.52±39.72[b]	684.00±53.37[b]	1217.01±74.37[b]	1418.92±53.40[b]	1415.42±55.06[b]	156±4[b]	7±1
12	33.3±0.16[a]	360.22±36.34[a]	593.40±48.82[a]	1091.86±68.03[a]	1255.03±48.85[a]	1262.14±50.37[a]	157±3[b]	7±1
13	33.7±0.14[a]	450.61±33.25[b]	662.18±44.68[b]	1171.81±62.26[b]	1354.84±44.71[b]	1368.12±46.10[b]	153±3[a]	8±1
22	33.5±0.38[a]	404.29±85.80[b]	644.95±115.28[b]	1077.46±60.64[a]	1173.08±115.35[a]	1269.35±18.93[a]	155±8[b]	8±4
33	31.2±0.27[b]	463.58±61.78[b]	679.90±83.02[b]	1330.38±115.68[b]	1396.83±83.06[b]	1419.52±85.64[b]	162±6[b]	6±2
ADL020								
11	32.8±0.17	415.29±38.99[b]	690.39±52.39[b]	1214.73±73.00	1348.31±52.41	1414.40±54.04[a]	149±4[a]	7±1
12	31.8±0.16	384.74±36.46[a]	599.66±48.99[a]	1185.12±68.26	1292.50±49.01	1353.12±50.54[b]	161±3[b]	7±1
13	32.9±0.17	449.44±39.04[b]	678.63±52.46[b]	1203.57±73.09	1296.26±52.49	1372.13±54.12[a]	152±4[a]	8±1
22	32.3±0.31	444.28±70.05[b]	650.76±94.12[b]	1200.56±131.15	1319.43±94.17	1289.85±97.10[b]	158±7[a]	7±3
23	35.1±0.25	399.20±58.30[a]	558.00±78.33[a]	1143.66±109.15	1304.72±78.37	1306.24±80.81[b]	164±6[b]	8±2
33	34.4±0.24	432.10±55.05[b]	739.88±73.97[b]	1118.58±103.06	1357.20±74.01	1345.72±76.31[b]	156±5[a]	8±2
ADL023								
11	33.9±0.17	439.15±44.23	690.08±56.26	1219.90±86.77	1445.82±65.47	1452.80±67.71	161±5[b]	7±2
12	35.3±0.04	372.27±12.04	630.86±15.31	1205.43±23.62	1376.30±17.82	1390.45±18.43	151±1[a]	7±1
13	34.2±0.05	387.60±14.15	651.20±17.99	1242.51±27.76	1396.03±20.94	1417.04±21.66	151±2[a]	8±1
23	37.2±0.18	408.66±46.78	651.20±59.50	1259.20±91.77	1464.66±69.25	1389.45±71.61	155±5[b]	6±3
ADL102								
11	35.6±0.09	408.34±22.06	670.30±29.41	1272.73±46.82	1434.30±34.85	1464.09±35.03	152±3	7±2
12	35.0±0.06	336.05±14.00	600.12±18.66	1192.11±38.67	1346.94±22.11	1356.30±22.23	152±2	7±1
13	34.6±0.06	413.36±13.33	670.22±29.17	1222.57±29.71	1412.79±29.23	1423.48±21.17	149±2	8±1
23	34.1±0.05	376.21±41.50	568.14±17.77	1108.04±28.29	1308.25±21.06	1327.21±42.17	158±1	8±1
33	31.7±0.17	405.31±38.55	650.85±51.38	1291.71±81.78	1394.84±60.88	1435.36±61.20	159±4	10±2
ADL176								
11	36.4±0.09	387.61±44.89	687.94±57.44	1312.49±79.92	1475.29±61.79	1383.63±30.74[a]	147±5[b]	8±2
12	35.1±0.18	374.69±13.11	643.05±16.78	1245.59±23.35	1407.54±18.05	1425.94±60.49[a]	151±1[bc]	7±1
13	32.5±0.05	415.92±45.01	631.78±57.59	1300.65±80.14	1453.48±61.96	1377.53±17.67[a]	157±5[c]	7±2
14	36.9±0.18	412.85±44.99	676.29±57.57	1219.56±80.11	1389.34±61.93	1407.67±60.66[a]	153±5[bc]	6±2
16	35.7±0.23	431.53±57.24	681.95±73.24	1086.85±101.92	1506.15±78.79	1541.28±60.63[c]	139±6[a]	5±3
25	33.0±0.20	344.36±51.39	610.36±65.76	1159.60±91.51	1361.83±70.75	1421.48±77.14[a]	156±6[c]	10±3
33	33.9±0.09	367.19±21.83	635.26±27.93	1195.11±38.87	1334.80±30.05	1394.88±69.26[a]	152±2[bc]	8±2
34	32.9±0.22	399.99±55.34	612.71±70.81	942.10±98.53	1249.95±76.17	1192.13±29.42[b]	157±6[c]	7±3
36	35.4±0.22	426.54±55.29	688.29±70.74	1311.52±98.44	1362.57±76.10	1421.18±74.57[a]	149±6[b]	8±3
44	35.6±0.16	388.09±39.87	659.45±51.01	1274.11±70.98	1437.14±54.88	1427.83±53.72[a]	152±4[bc]	7±2

D wt, 8 wt, 12 wt, 28 wt, 40 wt=Body weight at day old, 8, 12, 20, 28 and 40 weeks of age; Asm=Age at sexual maturity, Titre=SRBC titre. Different superscripts indicate significance at $P < 0.05$.

significantly associated with body weight at 40 weeks of age. Genotype 16 showed highest body weight at 40 weeks of age while genotype 11, 12, 13, 14, 25, 36 and 44 revealed moderate amount of body weight at this age. In contrast, birds with genotype 34 had lowest body weight at 40 weeks age, which was significantly ($P < 0.05$) different from other genotypes. Subsequently, birds with 34 genotypes should not be favored in the selection programme in order to its poor body mass.

Association with Other Traits

MCW007, ADL020, ADL023 and *ADL176* microsatellite were found to be significantly associated with age at sexual maturity whereas humoral immune response was observed to be non-significant with the microsatellites studied in the present endeavor (Table 5). In case of MCW007 microsatellite, genotype13 showed lowest age at sexual maturity (ASM) whereas other genotypes had higher ASM value, which was ranging from 155 to 162 days. For *ADL020,* four genotypes such as 11, 13, 22 and 33 revealed more or less similar length of ASM ranging from 149 to 158 days whereas genotypes 11 and 23 showed higher ASM which was found to be more than 163 days. Under *ADL023* microsatellite, genotype 12 and 13 had lowest ASM (151 days) than other genotypes while genotype 11 showed highest magnitude. The age at sexual maturity was not found to be significantly differed between genotype 11 and 23. In case of *ADL176* microsatellite, genotype 16 had lowest ASM (139 days) while 13, 25 and 34 showed highest age of sexual maturity by revealing significant differences ($P < 0.05$) from other genotypes.

Discussion

The chromosomal intersection of chicken genome explores that chromosome 1, 2 and 5 have been the hot point of genes for muscle growth, fatness and disease tolerance traits. In fact, chromosome 1 carries genes regulating growth like peroxisome proliferative activated receptor-α (*PPARA*), insulin-like growth factor- I (*IGF-I*), high mobility group I-C (*HMGIC*), lactate dehydrogenase B (*LDHB*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPD*), *TPT1* (Growth regulated protein), *MYH6* (Myosin heavy chain), *GAPDH* etc. whereas chromosome 2 bears *CDH2* (Cadherin 2), Collectin, *HAS2* (Carbohydrate metabolism), *LOC421012* (Metabolic enzyme) etc. and chromosome 5 carries *IGF2* (Insulin like growth factor 2), *GALNTL1* (Galactosamine activity), EGF-like 2 (Cartilage development) etc. Genes regulating disease resistance traits are located on chromosome 1 (*TRAI* for heat shock protein), chromosome 2 (Interleukin 6 for innate immunity) etc. while chromosome 5 carries genes like *LOC395381* (Ovomucin) etc. for reproductive functions etc. Besides, chromosome 1, 2 and 5 have been very much important as these chromosomes bear QTLs for muscle growth, fatness and disease tolerance like suppressor of cytokine signaling 2 (Gene ID 395219), suppressor of cytokine signaling 2 (Gene ID 423557), *LOC417950,*

LOC418051, LOC417978, LOC771315, LOC418668, interferon gamma, gremlin1, insulin, *TTLL12* (Gene ID 418227), *LOC428082* and *ASB9* genes (www.ncbi.nlm. nih.gov; Abasht *et al.,* 2006; Zhou *et al.,* 2006). Each marker was tested on all the samples of six crossbred chicken populations.

One to one correspondence in the form of significance between the microsatellites with phenotypes like body weight, ASM and humoral response may be the informative indicator for elucidating QTL and microsatellite relationship. The degree of relationship between microsatellites and growth traits may open new vistas for exploring markers with respect to growth traits in chicken lines, where production, reproduction as well as health status primarily depend on the body weight of birds.

All the five microsatellites used in this study were polymorphic and were distributed across the population. The genotypic proportions were distributed from low to moderate. However, the presence of one animal under a specific genotype group was not included in the association study. The allelic frequencies of all microsatellites except *ADL176* were varied from 20 to 50%. 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 the form of heterozygotes. However, the higher informativeness of microsatellites are well known and our study also reciprocate the same trend. Osman *et al.* (2004) used microsatellites for studying genetic variability in the Oh-Shamo and its related chicken breeds.

The variability of microsatellites which are located on the specific chromosomes where QTLs for growth traits are present were exploited to obtain certain relationship with growth parameters. The genetic principle of significant association of microsatellites and traits is possibly the phenomenon of linkage. There is a genetical fact that if microsatellite is linked with certain chromosomal location regulating phenotypes, it will specifically be observed in terms of significant association. Our study corroborate that *MCW007, ADL020, ADL102* and *ADL176* microsatellite were significantly correlated with body weights at different age groups in chicken. Certain genotypes were having higher body weight over others whereas some genotypes were showing poor growth. Out of all the studied microsatellites, *MCW007* revealed significant association with body weight at all the age groups. Our findings were conformed to the reports mentioned by Jennen *et al.* (2004) delineating significant correlation of microsatellites with body weight at 10 weeks of age. Van Kaam *et al.* (1998, 1999) indicating the presence of QTL for body weight at the age of 48 day located on chromosome 1 whereas by Ikeobi *et al.* (2002) reported a QTL for abdominal fatness in the same chromosome. Tuiskula-Haavisto *et al.* (1998) exploited microsatellite variation for elucidation of markers for egg quality traits in chicken. Nonetheless, our study suggests that microsatellite variability may be the good indicator for isolating chromo-

somal region controlling QTLs with respect to biological traits.

All the microsatellites except *ADL102* showed significant association with age at sexual maturity. Certain genotypes were showing lower ASM while others were having a moderate to high ASM. Our genome scan study delineate that these microsatellite may have linkage with QTLs for ASM. The present findings would have certain value with respect to total lifetime egg production. Lower ASM augments higher productivity leading to better lifetime production and of higher economic importance. Particularly, crossbred population was evolved for better productivity and introgression of economically important genes like immune competence in the population. The main problem of layer population is that higher the production, higher is the stress and higher the mortality. Hence, recently, immune competence in the form of humoral response has been the added advantage for layer industry. Shukla *et al.* (1996) and Jai Sunder *et al.* (2004) outlined the status of humoral response to SRBC in native chickens and white leghorns. Concomitantly, this trait has been one of the important selection criteria in some of the pure line layer population in India. However, physical mapping will help to perform finer mapping study for locating microsatellites and QTLs on the specific chromosome. Our initial approach for microsatellite and trait linkage disequilibrium would be one of the new dimensions to explore genetic markers for economically important traits in chicken population.

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