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Short Communication

LEI0258 microsatellite variability and its relationship to *B-F* haplotypes in Brazilian (blue-egg Caipira) chickens

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Abstract

A total of 149 chickens from two different sources (one non-commercial, the other commercial) was tested for variability of the LEI0258 microsatellite. Fifty- three genotypes, explainable by 15 alleles, were found. There are clear allele and heterozygosity differences between the two samples. One of them was simultaneously studied for the MHC *B-F* haplotypes. Strong genetic disequilibrium was observed between the variants of the two systems, therefore providing a cheap alternative for MHC genotyping.

Key words: microsatellite, LEI0258, *B-F* haplotypes, Brazilian chickens.

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Microsatellites (also called short tandem repeats or STRs) are moderately repetitive DNA sequences with small core sizes (di-, tri- or tetranucleotides mainly) that have been widely used to map genes, characterize inbred lineages, associate normal with pathological conditions, and uncover interpopulation differences. Examples in chickens are the studies of Kaiser *et al.* (2000), Wardecka *et al.* (2002), and Yunis *et al.* (2002), in which they have been used to characterize broiler populations, and were associated with egg production, quality traits, and antibody responses. McConnell *et al*. (1999) described one such tetranucleotide repeat system (LEI0258), located in chicken's microchromosome 16. Molecular markers in this chromosome are of special interest, since the major histocompatibility B complex also occurs there (Wain *et al*., 1998). More specifically, this microsatellite is placed near the *B-F*/*B-L* region. Genes in this DNA segment codify molecules which are responsible for the presentation of endogenous (B-F) and exogenous (B-L) antigens to the effector cells, therefore starting the whole chain of the immune response (Abbas *et al.*, 2000). It was shown that alleles or haplotypes in this region can influence the resistance or susceptibility to many avian diseases. For instance, haplotype B21 confer resistance, B2, B6, and B14 moderate resistance and B19 susceptibility to Marek Disease (Kaufman, 2000).

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We recently investigated the *B-F* region of a Brazilian (blue-egg Caipira) breed. Twenty-three sequences were obtained, of which at least 10 were novel, confirming these animals as potential sources for genes resistant to diseases and adverse climate, temperature, and food conditions. Fourteen different haplotypes were identified (Lima-Rosa *et al*., 2004). It is important to verify whether the LEI0258 variants are associated with these haplotypes, since they could provide markers that are detectable with an easier (and cheaper) laboratory method than sequencing. This was the objective of the present investigation. In addition, we wanted to verify whether different Caipira populations would present significant genetic heterogeneity.

Two populations were studied. The first was represented by 100 blue-egg Caipira chickens. The word Caipira derives from the Tupi-Guarani and means "habitant of the field." It is applied to domestic chickens in general, bred freely in the courtyards of rural or urban houses. An interesting characteristic of some of these chickens is that they may oviposite blue eggs. This trait appeared first in Chilean Araucana chickens, that around 1880 expanded to Brazil and crossed with Caipira, originating then the blue-egg Caipira chickens (Albino *et al.*, 2001; M.N. Sales, personal communication). Blue eggs were collected in farms from Dois Lageados county, state of Rio Grande do Sul, Brazil.

They were then placed in an incubator machine (Petersime Industrial SA, Urusanga, SC, Brazil) until hatching.

The other population was constituted by 49 animals from the Paraíso Pedrês commercial line, purchased from the Aves do Paraíso farm, located at km 10 of the Romildo Prado highway, in Itatiba, state of São Paulo, Brazil. This broiler breed was selected by crossing Caipira chickens with selected standard races, to obtain commercially valuable and homogeneous lineages (Ramos, 1995). Eleven of them were then intercrossed, leading to this Paraíso Pedrês breed. Peripheral blood was obtained from these and the other chicks using 0.5% EDTA as anticoagulant.

Genomic DNA was extracted from the birds' erythrocytes using Sambrook *et al*. (1989) standard method. Amplification by the polymerase chain reaction (PCR) was performed as described by McConnell *et al*. (1999), with some adjustments, from total DNA. The primers used were those described by these authors: forward (5'-CACGCAGCAGAACTTGGTAAGG-3') and reverse (5'-AGCTGTGCTCAGTCCTCAGTGC-3'). The mixtures for the PCR reactions, and reagent concentrations, were as follows: 50 ng of DNA, 2.5 μL of 10x buffer (100 mM tris-HCl, pH 8.3, 500 mM KCl, 15 mM Mg Cl_2), 2.5 μL of PCRx Enhancer System buffer (Invitrogen Life Technologies), 2 μL of dNTP mix (1.25 mM of dATP, dCTP, dGTP, and dTTP), one μM of each primer, 0.25 units of Platinum *Taq* DNA polymerase (Invitrogen Life Technologies) and distilled water to obtain a final volume of 25 μL. The amplification conditions consisted of a 1-minute initial denaturation at 96 °C, followed by 35 1-minute denaturation cycles at 96 °C, 30 s annealing at 54 °C, 30 s extension at 72 °C, and 3-minutes of final extension at 72 °C. The PCR products were analyzed by vertical electrophoresis in 7% non-denaturing polyacrilamide gel (Lahiri *et al*., 1997).

The *B-F* haplotype variability in the blue-egg Caipira sample had been previously evaluated through sequencing and cloning techniques described in Lima-Rosa *et al*. (2004). Briefly, the primers were those indicated by Li *et al*. (1997), the PCR conditions those described by Li *et al*. (1997) and Ennis *et al*. (1990), and the sequences were obtained using a MegaBACE 1000 machine. As for the cloning studies, they were performed using the Topo TA Cloning Kit for Sequencing (Invitrogen Life Technologies).

The alleles were identified by their sizes, and allele frequencies were determined by gene counting. Fit to the Hardy-Weinberg equilibrium was determined by the Markov chain method (Guo and Thompson, 1992) using version 2000 of the Arlequin program (Schneider *et al*., 2000). The interpopulation comparison of the allele frequencies was performed by the chi-square test (Roff and Bentzen, 1989), while the LEI0258/*B-F* linkage disequilibrium was also determined by the Arlequin program, with D' (the degree of departure from equilibrium) manually calculated as described by Lewontin (1988).

Fifty-three genotypes were found, 19 only in the blue-egg Caipira and 25 only in Paraíso Pedrês, nine, therefore, occurring in the two populations. Genotype 217/217 was the most frequent (30%) in the blue-egg Caipira chickens, followed by 217/325 (9%). In the Paraíso Pedrês sample almost all genotypes varied from 2 to 4%, the exception being 205/205, with a frequency of 8% (detailed information available on request). Allele frequency information is given in Table 1. A total of 15 alleles were observed in the 149 chickens tested, their sizes varying between 205 and 457 bp. Nine alleles were present in the two samples. Alleles *313*, *337*, and *421* were only found among the blue-egg Caipira chickens, while *373*, *385*, and *457* occurred exclusively in the Paraíso Pedrês sample. There are clear allele differences between the two populations ($\chi^2 = 111.4$; $p < 0.0001$). The three most frequent alleles in the blue-egg Caipira sample were *217* (42%), *325* (16%), and *265* (15%), while for Paraíso Pedrês they were *265* (16%), *397* (15%), and *205* (14%). The observed heterozygosity was 50% in blue-egg Caipira and 75% in Paraíso Pedrês chickens. Good genotype frequency fit to the Hardy-Weinberg predictions was obtained in the Paraíso Pedrês (p:0.207) but not in the blue-egg Caipira ($p < 0.001$) samples.

The number of alleles observed in our material is high (15); the study of other microsatellites by other authors in non-commercial chickens detected, at most, 11 alleles per locus (Takahashi *et al*., 1998; Zhou and Lamont, 1999; Wimmers *et al*., 1999, 2000; Marle-Köster and Nel, 2000).

Table 1 - LEI0258 allele frequencies in two samples of Brazilian chickens.

Allele[1]	Blue-egg Caipira (%)	Paraíso Pedrês (%)
205	8.0	14.3
217	42.0	10.2
229	2.0	3.1
265	15.0	16.3
277	8.0	7.1
301	3.5	2.0
313	0.5	-
325	16.0	4.1
337	2.5	-
373	-	8.2
385	-	5.1
397	0.5	15.3
409	1.5	4.1
421	0.5	-
457	-	10.2
Total	100	100

[1]Size in number of base pairs.

This could be due to the fact that LEI0258 is located in a region where genes of the major histocompatibility B complex also occur, which could be under the influence of diversifying selection. This type of selection generates diversity not only in the alleles under its influence, but also in adjoining regions (the so-called hitchhiking effect). Regardless of its causes, the LEI0258 variability could be quite useful in the investigation of several academic and applied problems.

Our samples derived from two distinct populations, one which was subjected to artificial selection for meat production and quality, while the other was from animals freely raised in farms, without this type of intentional breeding. The number of alleles detected in each (12), however, was the same. Allele distribution and degree of heterozygosity, on the other hand, were different. This is not surprising, since they originated from widely separated (about one thousand kilometers) populations. In the absence of detailed information about their founders and of experimental data, we can only speculate about the factors which may explain these results. Founder or sampling effects, sexual selection (unequal contribution of genetically different males in the two groups), or even some adaptive advantage of given alleles may have influenced these distributions.

Information about the association between the LEI0258 alleles and *B-F* haplotypes in the blue-egg Caipira chickens is provided in Table 2. Eight alleles (*205*, *229*, *301*, *313*, *337*, *397*, *409*, *421*) showed association with just one, two (*277*, *325*) with two, and other two (*217*, *265*) with three *B-F* haplotypes. *BF*CC14* was the only haplotype associated with two LEI0258 alleles (*313* and *337*). Linkage disequilibrium was quite high ($\chi^2 = 637.3$; $p < 0.0001$).

The strong association between LEI0258 alleles and *B-F* haplotypes has important theoretical and practical applications. First, the microsatellites may provide indirect information about the population distribution of *B-F* markers. But perhaps of higher value is the fact that in 67% of the cases there was a direct correspondence between a given LEI0258 allele and a specific *B-F* haplotype, providing an easier method for determination of the latter (*B-F* typing generally requires sequencing techniques). Even in the case where more than one *B-F* haplotype is associated with a LEI0258 allele, allele specific (PCR-SSP; Polymerase Chain Reaction with Sequence Specific Primers) selective typing can be performed, thus considerably reducing the cost of *B-F* genotyping. Of course, our sample was limited in size (100 animals), and different associations can be found in other breeds. But the results are valuable in that they draw attention to a source of variation not previously widely explored that can be used in a variety of ways.

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Table 2 - Association between the LEI0258 alleles and *B-F* haplotypes in blue-egg Caipira chickens.

LEI0258 allele	*B-F* haplotype[1]	Frequency	
		N	%
205	*BF*CC1*	16	8.0
217	*BF*CC3*	21	10.5
	*BF*CC4*	43	21.5
	*BF*CC15*	19	9.5
229	*BF*CC5*	4	2.0
265	*BF*CC6*	2	1.0
	*BF*CC7*	17	8.5
	*BF*CC8*	11	5.5
277	*BF*CC9*	4	2.0
	*BF*CC16*	12	6.0
301	*BF*CC10*	7	3.5
313	*BF*CC14*	1	0.5
325	*BF*CC13*	20	10.0
	*BF*CC17*	13	6.5
337	*BF*CC14*	5	2.5
397	*BF*CC2*	1	0.5
409	*BF*CC11*	3	1.5
421	*BF*CC12*	1	0.5
Total		200	100

[1]*B-F*CC15*, *B-F*CC16*, and *B-F*CC17* had not been described by Lima-Rosa *et al*. (2004), but were identified with essentially the same methods described by these authors.
N: number of chromosomes examined.

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