

GENETICS AND MOLECULAR BIOLOGY

MHC-*B* variation in maternal and paternal synthetic lines of the Argentinian Campero INTA chicken

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ABSTRACT The Campero-INTA chicken of Argentina was developed to provide a robust bird that can survive under Argentinian pasture conditions with no significant additional nutrition, producing a source of animal protein for small producers or low-income families. In previous work, we described the AH paternal line of Campero and its Major Histocompatibility Complex B region (MHC-B) variation. In this work we analyzed the three remaining synthetic lines used to produce the Campero-INTA production bird: lines AS, A, and E. Because of the association between variation within the MHC of chickens and disease resistance, MHC variation

within this breed is of particular interest. MHC variability within the lines used to produce the Campero-INTA chicken was examined using a 90 SNP panel encompassing the chicken MHC-*B* region plus the VNTR, LEI0258, located within the chicken MHC. Across all 4 lines 12 haplotypes were found, with 7 of these being previously reported in North America/European breeds, reflecting the original breed sources for these birds. Three Campero unique haplotypes were found, 2 of which likely originated from MHC recombination events. MHC-B variation for all lines involved with production of the final Campero-INTA bird has now been determined.

Key words: MHC variation, MHC haplotypes, LEI0258, campero chicken

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INTRODUCTION

The Campero synthetic line of chickens was developed by INTA (*Instituto Nacional de Tecnología Agropecuaria*) in the 1980s at the Pergamino experimental research station in Buenos Aires, Argentina. It was developed to provide a slow growing poultry variety well-adapted to free-range pasture production conditions in Argentina, with no significant feed input requirements. These birds are multicolored (all colors are accepted except for white to distinguish them from commercial broilers), with 110 eggs produced per year (Revidatti et al. 2005). Campero chickens have a body weight of 3.1K for males and 2.2K

for females at 84 d of age (Canet et al., 2014). Approximately 100,000 Camperos chicks are provided annually to multiple small producers throughout Argentina. (Canet, personal communication).

The breed was developed from crosses of common North America/European derived breeds including Barred Plymouth Rock, Cornish Red (one of the progenitor breeds of the modern broiler) and Rhode Island Red. From this synthetic population base, 4 parental lines were ultimately developed. These include 2 sire lines (AH and AS) and 2 dam lines (A and ES). Lines were developed for more than 20 generations (Canet, 2003). The maternal lines (A and ES) both originated from Cornish Red and Rhode Island Red breeds. The maternal lines are crossed to produce a hybrid dam (C), which is then mated with either AH or AS sire lines to produce the final commercial production bird (Figure 1).

The Major Histocompatibility Complex (MHC) region of the chicken genome contains many genes which

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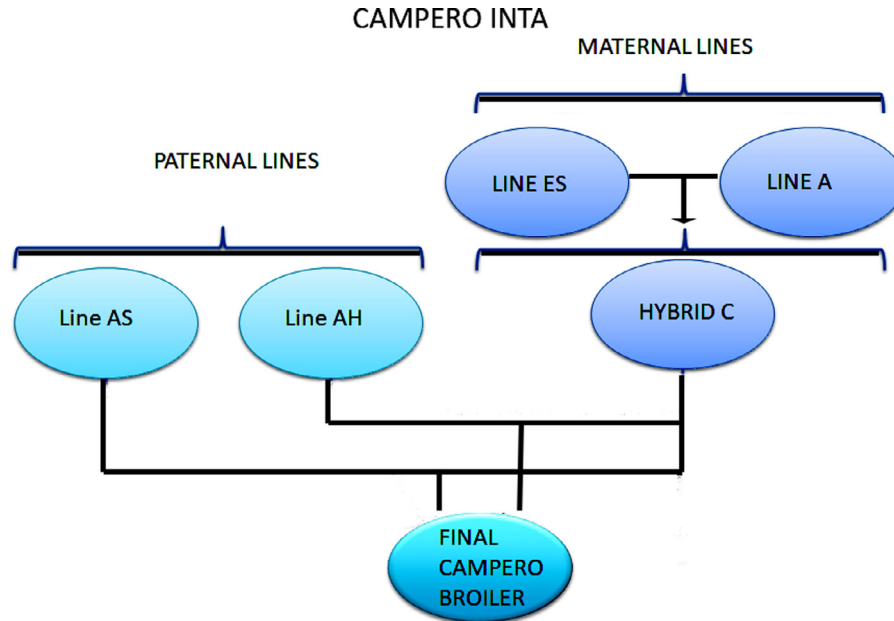


Figure 1. Campero INTA chickens and the lines used for the final product. Either sire line (AS or AH) are used for crossing with Hybrid C (ES x A) dam.

encode proteins involved with immunity. Numerous studies have shown that variation within the chicken MHC region has a very strong influence on disease resistance for multiple pathogens (review by Miller and Taylor, 2016). Variability of MHC is known to be an important component for disease resistance (Kaufman, 2018). Variation within the MHC is likely to have an important immunological role in environments with multiple pathogen challenges or with limited vaccinations, particularly under the Campero pasture poultry production systems. The chicken MHC was initially identified as the B blood group system, and variation was detected by the use of B system specific serological reagents (Briles et al., 1950). MHC variability can now be detected utilizing DNA-based methods (Fulton 2020). The Variable Number of Tandem Repeats (VNTR) LEI0258 is located within the MHC and has been shown to be useful in detection of MHC haplotypes (Fulton et al., 2006). This marker has been used extensively in multiple global chicken populations and revealed much MHC diversity (Lima Rosa et al., 2005; Lwelamira et al., 2008a; Izadi et al., 2011; Han et al., 2013; Nikbakht et al., 2013; Guangxin et al., 2014; Nikbakht et al., 2015; Nikbakht and Esmailnejad, 2015; Mwambene et al., 2019; Mpenda et al., 2020; Haunshi et al., 2020a). Recently, a 90 SNP panel covering 240,000 bp of the MHC-B region (encompassing genes BG2 through CD1A1) was developed and used to define multiple haplotypes found in commonly utilized North America and Europe breeds (Fulton et al., 2016b). Many of these samples had previously defined ‘serological’ haplotypes and were thus useful as a link to serological B typing. Application of this MHC-B region SNP panel (MHC-BSNP) has been used for numerous chicken populations globally to identify MHC variation within different breeds and indigenous chickens (Fulton et al., 2016a,b, 2017; Nguyen-Phuc et al., 2016;

Hako Touko et al., 2015; Iglesias et al., 2019; Manjula et al., 2020; Tarrant et al., 2020; Haunshi et al., 2020a).

Previous work identified MHC-BSNP haplotypes within one of the Campero sire lines (line AH) (Iglesias et al., 2019). The work presented herein extends that initial study, adding additional information of the MHC-BSNP haplotypes, including LEI0258 alleles found, in the other 3 lines (A, AS, and ES) used to produce the Campero hybrid production birds.

MATERIALS AND METHODS

Genetic Lines

The AS paternal line is maintained at a population size of 200 females and 60 males. The 2 maternal lines are maintained with 120 females and 60 males. For this study, samples were obtained (in 2019) from each of the three lines with $n = 80$ for AS, $n = 45$ for A, and $n = 50$ for ES, for a total of 175. Samples were obtained from both males and females. All lines are routinely vaccinated against several diseases including Marek Disease, Infectious bronchitis, and Newcastle Disease.

Sample Description

Whole blood samples were collected in 1.5 mL microtubes with EDTA (Ethylenediaminetetraacetic acid) from brachial vein. Twenty μL of each sample was placed on FTA Elute cards (Millipore Sigma, Burlington, MA) and allowed to dry at room temperature. DNA was extracted from cards following manufacturer recommendation and resuspended in 200 μL of dH_2O for use in PCR. DNA from whole blood was extracted with salt and ethanol precipitation for LEI0258 typing.

Animals were raised in accordance to regulatory agency guidance (CICUAL Comité Institucional de cuidado y uso de animales de laboratorio), with blood sampling done following agency guidelines.

LEI0258 Microsatellite Genotyping

Amplification of LEI0258 locus was performed using primers developed by (McConnell et al., 1999), For: 5'-CACGCAGCAGAACTTGGTAAGG-3' and Rev: 5'-AGCTGTGCTCAGTCCTCAGTGC-3'. LEI0258 PCR was carried out using 100 ng of genomic DNA with 150 ng of each primer, 1.5 mM of MgCl₂ 0.2 mM of dNTPs and 0.3 units of TaqPol (Promega, US, Invitrogen, Brazil and Inbio Highway, Argentina) in the supplied buffer in a final volume of 20 μ L. The PCR reaction was performed in MultiGene OptiMax Lasergen Thermal Cycler, China, using the following program: 94°C for 5 min, 35 cycles of 95°C for 1 min, 63°C for 40 s, 72°C for 1 min, followed by 7 min extension at 72°C. Fragment sizes were determined from 3% agarose gel stained with ethidium bromide staining (10 mg/mL) using 100 bp marker (Promega, US) and visualized under UV light. DNA from known B21 serotype birds was used as a sizing control.

MHC-BSNP Genotyping

The SNP genotyping of the MHC region was done using a high-density SNP panel, as described by Fulton et al. (2016b), following the same protocol as given in Iglesias et al. (2019) using KASP chemistry (Semagn et al., 2014). For each SNP, PCR is performed independently (single-plex) with the two alleles having a different fluorescent label. The presence of each fluorescently labeled allele is detected as endpoint reads with a fluorescence plate reader, and genotype determined based on relative of levels of specific fluorescence. MHC-BSNP haplotypes (i.e., specific combination of alleles over 90 SNP in the MHC-B region) and LEI0258 allele sizes were identified for all samples.

Homozygotes were identified first, due to their homozygosity for all SNP. Heterozygotes were aligned with an existing relevant haplotype. The additional haplotype present within a heterozygote was determined by subtraction of the relevant homozygote haplotype SNP allele at each heterozygous site, with the alternate allele defining the novel haplotype. All haplotypes obtained were then compared with those previously defined in Fulton et al., 2016b, that is, the 'Standard' haplotypes. All haplotypes found in the Campero lines were aligned to determine if any may be explained by MHC recombination between other haplotypes found within the line, as described by Fulton et al., 2016b.

Statistical Analysis

Allelic frequencies for MHC-BNSP defined haplotype combinations, Hardy-Weinberg equilibrium, and Wright's F_{IS} were estimated using Genepop 4.7.5 (Rousset, 2008) software.

RESULTS AND DISCUSSION

MHC-BSNPs Haplotypes Found in Camperos Lines

LEI0258 allele size and BSNP haplotypes were determined on all 175 samples. The MHC-BSNP haplotypes with LEI0258 allele size, and their frequency found within each line are summarized in Table 1. Across the three lines (AS, ES and A), 11 haplotypes were found, and of these, 7 were the same as those previously identified as 'Standard' haplotypes, with four being unique to the Campero lines. The breed origin of these 'Standard' haplotypes is also provided in Table 1 and were reported previously in either heritage broilers, RIR or WPR (Fulton et al., 2016a), all breeds related to those used for the original development of these lines. The LEI0258 allele size detected was consistent with previous reports for these standard haplotypes.

Table 1. MHC-BSNP haplotypes with LEI0258 allele size and their frequencies in the four parental lines utilized to produce the hybrid Campero chicken.

BSNP Haplotype	LEI0258 size	Breed ¹	Line A	Line AS	Line ES	Line AH (2018) ²
BSNP-A08	357	RIR, WPR			0.17	
BSNP-D04	205	BRL, WL	0.21	0.01	0.46	0.01
BSNP-M01	307	RIR, WPR	0.17	0.16	0.21	0.05
BSNP-002	309	NH, RIR	0.07		0.15	
BSNP-Q01	193	BRL	0.01	0.09		0.42
BSNP-V03	381	BRL		0.16		
BSNP-V05	381	BRL	0.02	0.52		0.35
BSNP-Camp-H02	205			0.03		
BSNP-Camp-H04	nd					0.17
BSNP-Camp-H05	381		0.39	0.01		
BSNP-Camp-H06	381		0.13	0.01	0.01	
BSNP-Camp-H07	381			0.03		
Total No. Haplotypes		7	9	5	5	

Abbreviations: BRL, broiler; nd, unknown; NH, New Hampshire; R/JF, Red Jungle fowl; RIR, Rhode Island Red; WPR, White Plymouth Rock; WL, White Leghorn.

¹These haplotypes were previously reported in specific breeds.

²From Iglesias et al. (2019).

Haplotype information for the most recent (2018) sampling of the other sire line (AH) as described by Iglesias et al. (2019) was also included in Table 1 to allow the haplotype analysis to be extended to include all four lines utilized to produce the final Campero hybrid production bird. The addition of information from the previously reported AH line results in an increase of one MHC-BSNP haplotype, bringing the total to 12, with 7 being previously identified as ‘standard’ MHC-BSNP haplotypes, and 5 being unique for the Campero breed. BSNP-Camp-H01, H02 and H03 were found in the 2002 sampling of line AH but were not detected in the 2018 sampling. The novel BSNP-Camp-H04 was found in 2018 and attributed to an introgression of Fayoumi breed into the AH line (Iglesias et al., 2019). The number of haplotypes per line ranges from 5 to 9, similar to the 5 to 11 reported in heritage broilers (Fulton et al., 2016a). A decrease in MHC-BSNP haplotypes from 10 to 5 haplotypes between 2002 and 2018 was reported for the AH line, even though another line had been. The 6 lowest frequency haplotypes (freq.< 0.10) were lost in the AH line during the 16 yr between sampling. Samples for lines A, AS, and ES from previous generations were not available for testing and similar comparisons (Figure 2).

In all four of the lines, the MHC-BSNP frequencies show considerable variability, from a low of 0.01 for BSNP-D04 in lines AH and AS, BSNP-Q01 in line A, and BSNP-Camp05, and 06, to a high of 0.52 for BSNP-V05 in line AS. It would be expected that low frequency haplotypes such as novel recombinants could occur and then be lost due to sampling and small population size. Phenotypic trait association studies could be done to determine if there are selective advantages for specific haplotypes within these lines and their environmental challenge.

The four BSNP-Camp haplotypes with their unique MHC SNP allele combinations are shown in Figure 2. Close examination of these haplotypes following alignment to the other haplotypes found within the lines shows that 3 of these haplotypes appear to be due to a recombinational event. For BSNP-Camp-H07 both possible contributing parental haplotypes could be found as

it appears to be identical to BSNP-M01 from SNP MHCJ6 through SNP MHC-11 (9 SNPs) and then identical to BSNP-V05 from SNP MHCNew25 through MHC-178 (81 SNPs), thus showing likely identity to BSNP-V05 for consecutive 91% of the MHC. BSNP-Camp-H02 is identical to BSNP-D04 from SNP MHC-18 though MHC-178, covering 69% of the MHC-B region suggesting that this Campero unique haplotype arose by recombination involving haplotype BSNP-D04. Similarly, BSNP-Camp-H06 appears to have arisen by recombination as it shows identity with BSNP-V05 from SNP MHC-75 through MHC-178, covering 59% of the MHC. The other parental haplotype contributing to the latter 2 potential recombinants could not be identified within the populations and may have been lost over time. Each of these putative recombinations occurred in regions consistent with one of the recombination hotspots as defined by Fulton et al., 2016b. The occurrence of novel haplotypes due to recombination with the MHC-B region as identified by the BSNP pattern is not unexpected. Recombination within this region was estimated to occur at a rate of 7/2400 meiosis (Fulton et al., 2016b). Novel MHC haplotypes that can be explained by recombination have been seen in other chicken populations that were MHC haplotype-defined using this same MHC-BSNP panel (Fulton et al., 2016a; Tarrant et al., 2020; Manjula et al., 2020).

Lines A and ES were in Hardy-Weinberg equilibrium (HWE) while AS line was not ($P < 0.01$). For AS, the estimated Wright's FIS was 0.11 ($P = 0.003$) indicating a deficit of heterozygotes compared to that expected. Specifically, there was an excessive proportion of BSNP-V05 homozygotes. This may be due to selection for higher live weight, which could bias the use of specific birds or families for reproduction. The 3 lines were statistically different from each another based on BSNP haplotype frequencies ($P < 0.00001$).

Allele sizes for the VNTR LEI0258 located within the MHC (between SNP MHC-77 and MHC-79) were also obtained for each sample. Perfect consistency was found between each MHC-BSNP haplotype and the LEI0258 allele size. For the standard MHC-BSNP haplotypes,

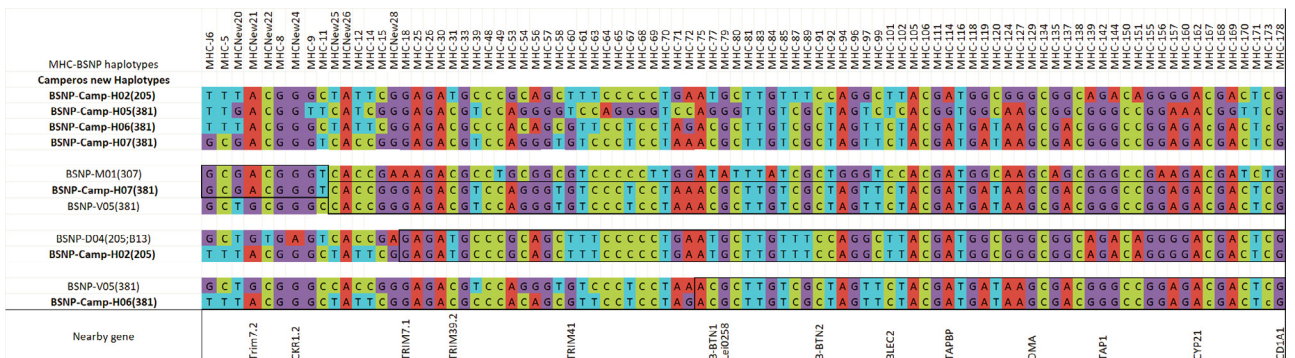


Figure 2. Haplotypes present in lines: A, AS and ES. Putative recombinants and their potential parental haplotypes are also provided. The SNPs panel in an excel file. Comparison of the haplotypes found in Campero’s haplotypes those previously described. Remarked with boxes is possible to see similar regions between Campero’s haplotypes and different ones already reported. Also some of the genes and the LEI0258 satellite was at the bottom of the figure.

the allele size was the same as that previously reported (Fulton et al., 2016b). For the novel haplotypes, BSNP-Camp-H05, H06, and H07 each have the same 381 bp allele, whereas BSNP-Camp-H02 has the 205 bp allele.

If MHC diversity within the Campero lines were being evaluated utilizing only the LEI0258 allele size information there would have been an underestimation of the number of haplotypes. While we found a total of 12 MHC-BSNP haplotypes, LEI0258 showed only 6 different allele sizes. Both BSNP-D04 and BSNP-Camp-H02 have the same LEI0258 allele of 205. The LEI0258 allele of 381bp is found for 5 haplotypes; BSNP-V03 and V05, plus BSNP-Camp-H05, H06 and H07. The MHC-BSNP panel interrogates many more sites than the single LEI0258 locus, thus providing additional information, and extending the detection of diversity. Furthermore, the use of a single marker (LEI0258) within the MHC-B region would not have identified the novel recombinants.

Previous Associations With Immune Response or Production Traits With the MHC-BSNP Haplotypes Found

Multiple disease and phenotype association studies have been done utilizing serologically defined B haplotypes. Since many of these are now also defined by MHC-BSNP haplotypes, this provides an opportunity to compare potential disease and production phenotypes for those MHC haplotypes found within the Campero chicken. The B13 serologically defined MHC haplotype has the BSNP-haplotype of BSNP-D04 with the LEI0258 allele size of 205 (Fulton et al., 2016b). The BSNP-D04(205) was found within all four of the Campero lines. The B13 haplotype is reported to show lower resistance to Marek's Disease Virus and higher coccidial oocyst counts than other haplotypes (Bacon 1987; Lillehoj et al., 1989). B13 was also shown to be associated with lower antibody titers and higher body weights (Dunnington et al., 1996). Other studies have reported an impact of B13 on several production related traits including livability and egg production (Briles and Allen, 1961). Studies with Tanzanian chicken ecotypes reported the LEI0258 allele size of 205 to be positively associated with a primary antibody response to Newcastle Disease vaccine (Lwelamira et al., 2008a). This same study reported the 307 allele, which is found with the BSNP-M01(307) haplotype, and is present in all four Campero lines, to be associated with a lower antibody response and positively associated with body weight (Lwelamira et al., 2008a).

Similar associations have been reported in the literature for other MHC-B alleles related to the MHC-BSNP haplotypes found in the Campero chicken lines. The BSNP-A08 (357) haplotype as found within the Campero chicken differs from BSNP-A04 only at the beginning of the MHC (near to the genes BG2, Trim 7.2 and CKR.1). These 2 haplotypes are identical for the remaining 90% of the downstream MHC as defined by the BSNP panel (Fulton et al., 2016b). Haplotype BSNP-

A04 is the serological B21 haplotype and thus BSNP-A08 is very similar to the B21 haplotype. B21 has been shown to provide strong protection against Marek's Disease Virus (Hansen et al., 1967; Briles et al., 1977; Bacon 1987). An association between B21 and lower mite infestation has also been documented (Owen et al., 2008). It should be noted that the specific loci within B21 that contribute to disease resistance is not known.

The LEI0258 allele 381 was found in 5 Campero haplotypes (BSNP-V03 and V05, BSNP-Camp-H05, H06 and H07). DNA from an individual from the AH line containing this same 381 allele provided sequence for exon 2 B-F region (Iglesias, unpublished) that was identical to C2V as defined by Livant et al. (2004); Livant et al., 2001; Livant and Ewald, 2005. This C2V allele was found to be associated with bodyweight (Ewald et al., 2007).

Because of the strong associations of MHC-B serologically defined alleles with disease resistance, variation within populations with potential high disease challenge is particularly valuable. Studies with the Campero-INTA chickens involving immune response associations and productive traits could lead to considerable improvements in disease resistance, productivity and overall livability. Future studies could include MHC associations and antibody response following vaccination, relationship between coccidia oocyst shedding or mite infestation levels and MHC, particularly since the MHC haplotypes identified within the Campero chickens have reported differences in responses to these pathogens. Furthermore, the breeding program continues to sustain the MHC types present to ensure that MHC variability is maintained for maximal opportunities for disease resistance in the hybrid progeny.

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DISCLOSURES

All the author declare that there is not any conflict of interest in the following article MHC-B variation in maternal and paternal synthetic lines of the Argentinian Campero INTA chicken

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