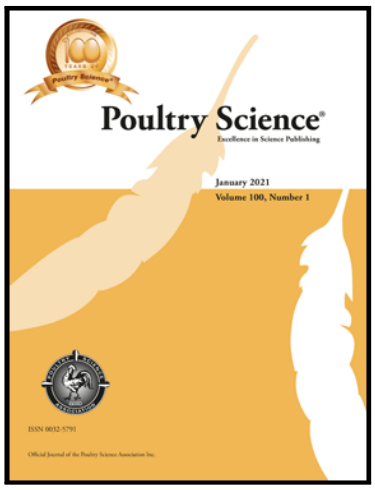

MHC-B variation in maternal and paternal synthetic lines of the Argentinian Campero INTA chicken

Gabriela M Iglesias , María P. Beker , Jose S. Remolins , Zulma E. Canet , José Librera , Horacio Cantaro , Daniel O. Maizon , Janet E. Fulton

PII: S0032-5791(21)00287-X
DOI: https://doi.org/10.1016/j.psj.2021.101253
Reference: PSJ 101253

To appear in: *Poultry Science*

Received date: 29 October 2020
Accepted date: 3 May 2021

Please cite this article as: Gabriela M Iglesias , María P. Beker , Jose S. Remolins , Zulma E. Canet , José Librera , Horacio Cantaro , Daniel O. Maizon , Janet E. Fulton , MHC-B variation in maternal and paternal synthetic lines of the Argentinian Campero INTA chicken, *Poultry Science* (2021), doi: https://doi.org/10.1016/j.psj.2021.101253

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

Gabriela M Iglesias [€], María P. Beker [€], Jose S. Remolins [€], Zulma E. Canet [±‡], José Librera [‡], Horacio Cantaro [€¥], Daniel O. Maizon [α] and Janet E. Fulton [Ω]

€ Universidad Nacional de Río Negro. Sede Alto Valle y Valle Medio. Escuela de Veterinaria y producción agroindustrial. Cátedra de Genética. Pacheco 460. Choele Choel, Rio Negro. 8360. Argentina. +5401150392164 [1]

± Universidad Nacional de Rosario. Facultad de Ciencias Veterinarias. Cátedra de Genética. Boulevard Ovidio Lagos y Ruta 33. Casilda. Santa Fe. Argentina.

‡ INTA Pergamino. Estación Experimental Agropecuaria "Ing. Agr. Walter Kugler". Av. Frondizi (Ruta 32) Km 4,5. Pergamino, Buenos Aires. Argentina.

¥ Estación Experimental Agropecuaria Alto Valle. Programa Nacional de Producción Animal. Ruta Nacional 22, Km 1190. Argentina.

α Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Anguil, Ruta Nacional 5 Km 580, Anguil, Argentina.

Ω Hy-Line International, P.O. Box 310 Dallas Center, IA 50063, USA

1. Corresponding author: giglesias@unrn.edu.ar

Short Title: MHC-B variation in Campero Chickens

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

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 four 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, two of which likely originated from MHC recombination events. MHC-B variation for all lines involved with production of the final Campero-INTA bird have now been determined.

INTRODUCTION

The Campero synthetic line of chickens was developed by INTA (*Instituto Nacional de Tecnología Agropecuaria*) in the 1980's 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 multi- colored (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 days 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, four parental lines were ultimately developed. These include two sire lines (AH and AS) and two dam lines (A and ES). Lines were developed for more than 20 generations (Canet and Terzaghi, 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 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., 2008; Izadi et al., 2011; Han et al., 2013; Nikbakht et al., 2013; Guangxin et al., 2014; Nikbakht et al., 2015; Wang et al., 2014; Nikbakht and Esmailnejad; 2015; Mwambene et al., 2019; Mpenda et al., 2020; Haunshi et al., 2020). 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; Iglesias et al., 2019; Manjula et al., 2020b; Nguyen-Phuc et al., 2016; Tarrant et al., 2020).

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 two 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. 20ul 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 200ul of dH_20 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 Institutional de cuidado y uso de animals de laboratorio), with blood sampling done following agency guidelines.

LEI0258 microsatellite genotyping

Amplification of LEI0258 locus was performed using primers developed by McConnell and co-workers (1999), For: 5´-CACGCAGCAGAACTTGGTAAGG-3´ and Rev: 5'-AGCTGTGCTCAGTCCTCAGTGC-3´. LEI0258 PCR was carried out using 100ng of genomic DNA with 150 ng of each primer, 1.5mM of $MgCl_2$ 0.2mM 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 µl. The PCR reaction was performed in MultiGene™ OptiMax Lasergen Thermal Cycler, China, using the following program: 94ºC for 5 min, 35 cycles of 95ºC for 1 min, 63ºC for 40 s, 72ºC for 1 min, followed by 7 min extension at 72ºC. Fragment sizes were determined from 3% agarose gel stained with ethidium bromide staining (10mg/ml) using 100 bp marker (Promega, US) and visualized under UV light. DNA from known B21 serotype birds was used as a sizing control.

141 ***MHC-BSNP genotyping***

142 The SNP genotyping of the MHC region was done using a high-density SNP panel, as
143 described by Fulton et al., 2016, following the same protocol as given in Iglesias et al.,
144 (2019) using KASP chemistry (Semagn et al., 2014). For each SNP, PCR is performed
145 independently (single-plex) with the two alleles having a different fluorescent label. The
146 presence of each fluorescently labelled allele is detected as endpoint reads with a
147 fluorescence plate reader, and genotype determined based on relative of levels of specific
148 fluorescence. MHC-BSNP haplotypes (ie specific combination of alleles over 90 SNP in the
149 MHC-B region) and LEI0258 allele sizes were identified for all samples.

150 Homozygotes were identified first, due to their homozygosity for all SNP. Heterozygotes
151 were aligned with an existing relevant haplotype. The additional haplotype present within a
152 heterozygote was determined by subtraction of the relevant homozygote haplotype SNP allele
153 at each heterozygous site, with the alternate allele defining the novel haplotype. All
154 haplotypes obtained were then compared with those previously defined in Fulton et al.,
155 2016a, i.e. the 'Standard' haplotypes. All haplotypes found in the Campero lines were aligned
156 to determine if any may be explained by MHC recombination between other haplotypes
157 found within the line, as described by Fulton et al., (2016a).

158

159 ***Statistical analysis***

160 Allelic frequencies for MHC-BNSP defined haplotype combinations, Hardy-Weinberg
161 equilibrium, and Wright's F_{IS} were estimated using Genepop 4.7.5 (Rousset 2015) software.

162

163

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., 2016), 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.

Haplotype information for the most recent (2018) sampling of the other sire line (AH) as described by Iglesias and co-workers (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-BNSP 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 5-9, similar to the 5-11 reported in heritage broilers (Fulton et al., 2016a). A decrease in MHC-BSNP haplotypes from 10 to five haplotypes between 2002 and 2018 was reported for the AH line, even though another line had been. The six lowest frequency haplotypes (freq.< 0.10) were lost in the AH line during

188 the 16 years between sampling. Samples for lines A, AS and ES from previous generations
189 were not available for testing and similar comparisons.

190 In all four of the lines, the MHC-BSNP frequencies show considerable variability, from a low
191 of 0.01 for BSNP-D04 in lines AH and AS, BSNP-Q01 in line A, and BSNP-Camp05, and
192 06, to a high of 0.52 for BSNP-V05 in line AS. It would be expected that low frequency
193 haplotypes such as novel recombinants could occur and then be lost due to sampling and
194 small population size. Phenotypic trait association studies could be done to determine if there
195 are selective advantages for specific haplotypes within these lines and their environmental
196 challenge.

197 The four BSNP-Camp haplotypes with their unique MHC SNP allele combinations are shown
198 in Figure 2. Close examination of these haplotypes following alignment to the other
199 haplotypes found within the lines shows that three of these haplotypes appear to be due to a
200 recombinational event. For BSNP-Camp-H07 both possible contributing parental haplotypes
201 could be found as it appears to be identical to BSNP-M01 from SNP MHCJ6 through SNP
202 MHC-11 (9 SNPs) and then identical to BSNP-V05 from SNP MHCNew25 through MHC-
203 178 (81 SNPs), thus showing likely identity to BSNP-V05 for consecutive 91% of the MHC.
204 BSNP-Camp-H02 is identical to BSNP-D04 from SNP MHC-18 though MHC-178, covering
205 69% of the MHC-B region suggesting that this Campero unique haplotype arose by
206 recombination involving haplotype BSNP-D04. Similarly, BSNP-Camp-H06 appears to have
207 arisen by recombination as it shows identity with BSNP-V05 from SNP MHC-75 through
208 MHC-178, covering 59% of the MHC. The other parental haplotype contributing to the latter
209 two potential recombinants could not be identified within the populations and may have been
210 lost over time. Each of these putative recombinations occurred in regions consistent with one
211 of the recombination hotspots as defined by Fulton et al., 2016b. The occurrence of novel
212 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., 2016b; Tarrant et al., 2019; 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 three 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, 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-BNSP 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 LEIO258 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. 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., 2008).

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 two 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 (Bacon 1987; Briles et al., 1977; Hansen et al., 1967). An association between B21 and lower mite infestation has also been documented (Owen et al., 2009). It should be noted that the specific loci within B21 that contribute to disease resistance is not known.

The LEI0258 allele 381was found in five 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. 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.

Acknowledgements

We want to thank all Hyline lab collaborators. The article was funded by PI-A-498 and PI A-805) "Genotipos del Complejo Mayor de Histocompatibilidad y su asociación con características productivas y de respuesta inmune en pollos parrilleros" from Universidad Nacional de Río Negro.

313 References

314 Ashwell, C. 2017. Insights from long term selection of White Leghorns for high and low
315 antibody response. Prestage Department of Poultry Science Nc State University. Genetic
316 Preservation Summit: Putting the Pieces Together PROCEEDINGS. May 24-25, 2017
317 Alberta Chicken Producers Poultry Technology Centre University of Alberta South
318 Campus Edmonton, Alberta, Canada.

319 Bacon, L. D. 1987. Influence of the Major Histocompatibility Complex on disease resistance
320 and productivity. Poult. Sci., 66:802-811.

321 Boonyanuwat, K., S. Thummabutra, N. Sookmanee, V. Vatchavalkhu, and V. Siripholvat.
322 2006. Influences of major histocompatibility complex class I haplotypes on avian
323 influenza virus disease traits in Thai indigenous chickens. Anim. Sci. J. 77:285–289.

324 Briles, W.E., and C. P. Allen. 1961. The B blood group system of chickens. II. The Effects
325 of genotype on livability and egg production in seven commercial inbred lines. Genetics
326 46:1273-1293.

327 Briles, W., W. McGibbon and M. Irwin. 1950. On multiple alleles effecting cellular antigens
328 in the chicken. Genetics 35:633-652.

329
330 Briles, W.E., H.A Stone and R.K Cole. 1977. Marek's disease: Effects of the B
331 histocompatibility alloalleles in resistant and susceptible chicken lines. Science 195:193-
332 195.

333 Canet, Z., and A. Terzaghi. 2003. Pollo Campero INTA. Idia XXI Available at
334 http://www.produccion-animal.com.ar/produccion_aves/produccion_avicola/18-
335 pollo_campero.pdf

336 Canet, Z. E., S.A. Advínculo, A.C. Sciutto, J.E. Librera, A.M.Dottavio, R.J. Di Masso. 2014.
337 Body conformation and slaughter characters in males and females of two experimental
338 three-way hybrids of free-range chickens. Revista Ciencias Veterinarias, Vol. 16, N° 1,
339 2014 (ISSN 1515-1883)

340 Dunnington, E. A., W. E. Briles, R. W. Briles, and P. B. Siegel. 1996. Immuno-
341 responsiveness in Chickens: Association of Antibody Production and the B System of the
342 Major Histocompatibility Complex. Poult. Sci. 75:1156–1160.

343 Ewald, S. J., X. Ye, S. Avendano, S. McLeod, S. J. Lamont, and J. C. M. Dekkers. 2007.
344 Associations of BF2 alleles with antibody titers and production traits in commercial pure
345 line broiler chickens. Anim. Genet. 38:174–176.

346 Fulton, J. E., H. R. Juul-Madsen, C. M. Ashwell, A. M. McCarron, J. A. Arthur, N. P.
347 O'Sullivan, and R. L. Taylor. 2006. Molecular genotype identification of the Gallus
348 gallus major histocompatibility complex. Immunogenetics 58:407–421.

349 Fulton, J. E., A. R. Lund, A. M. Mccarron, K. N. Pinegar, D. R. Korver, H. L. Classen, S.
350 Aggrey, C. Utterbach, N. B. Anthony, and M. E. Berres. 2016a. MHC variability in
351 heritage breeds of chickens. Poult. Sci. 95:393–399.

352 Fulton, J. E., A. M. McCarron, A. R. Lund, K. N. Pinegar, A. Wolc, O. Chazara, B.
353 Bed'Hom, M. Berres, and M. M. Miller. 2016b. A high-density SNP panel reveals
354 extensive diversity, frequent recombination and multiple recombination hotspots within
355 the chicken major histocompatibility complex B region between BG2 and CD1A1.
356 Genet. Sel. Evol. 48:1

357 Fulton, J. E., M. E. Berres, J. Kantanen, and M. Honkatukia. 2017. MHC-B variability within
358 the Finnish Landrace chicken conservation program. Poult. Sci. 96:3026–3030.

359 Fulton, J. E. 2020. Advances in methodologies for detecting MHC-B variability in chickens.
360 Poult. Sci. 99:1267–1274.

361 Guangxin, E., R. Sha, S. Zeng, C. Wang, J. Pan, and J. Han. 2014. Genetic variability,
362 evidence of potential recombinational event and selection of LEI0258 in chicken. Gene
363 537:126–131

364 Hako Touko, B. A., C. T. Keambou, J.-M. Han, C. Bembidé, R. A. Skilton, M. Ogugo, Y.
365 Manjeli, S. Osama, C.-Y. Cho, and A. Djikeng. 2015. Molecular typing of the major
366 histocompatibility complex B microsatellite haplotypes in Cameroon chicken. Animal
367 Genetic Resources/Ressources Génétiques Animales/Recursos Genéticos Animales, 56,
368 47-54. doi: 10.1017/s2078633614000538

369 Han, B., L. Lian, L. Qu, J. Zheng, and N. Yang. 2013. Abundant polymorphisms at the
370 microsatellite locus LEI0258 in indigenous chickens. Poult. Sci. 92:3113–9

371 Hansen M. P. , J. N. Van Zandt, and G. R. J. Law. 1967. Differences in susceptibility to
372 Marek's disease in chickens carrying two different B locus blood group alleles. Poult.
373 Sci. 46; 1268 (abs).

374 Haunshi, S., D. Devara, K. Ramasamy, R. Ullengala, and R. N. Chatterjee. 2020a. Genetic
375 diversity at major histocompatibility complex and its effect on production and immune
376 traits in indigenous chicken breeds of India. Arch. Anim. Breed 63:173–182.

377 Hunt, H. D., S. Jadhao, and D. E. Swayne. 2010. Major histocompatibility complex and
378 background genes in chickens influence susceptibility to high pathogenicity avian
379 influenza virus. Avian Dis. 54(1 Suppl):572-5. doi: 10.1637/8888-042409-ResNote.1.
380 PMID: 20521696

381 Iglesias, G. M., Z. E. Canet, H. Cantaro, M. C. Miquel, J. E. Melo, M. M. Miller, M. E.
382 Berres, and J. E. Fulton. 2019a. Mhc-B haplotypes in "Campero-Inta" chicken synthetic
383 line. Poult. Sci. 98:5281–5286.

384 Izadi, F., C. Ritland, and K. M. Cheng. 2011. Genetic diversity of the major
385 histocompatibility complex region in commercial and noncommercial chicken flocks
386 using the LEI0258 microsatellite marker. Poult. Sci. 90:2711–2717.

387 Kaufman, J. 2018. Generalists and Specialists: A New View of How MHC Class I Molecules
388 Fight Infectious Pathogens. Trends Immunol. 39.

389 Lillehoj, H. S., M.D. Ruff, L.D. Bacon, S.J. Lamont and T.K. Jeffers. 1989. Genetic control
390 of immunity to *Eimeria tenella*. Interaction of MHC genes and non-MHC linked genes
391 influences levels of disease susceptibility in chickens. Vet. Immunol. Immunopath.
392 20:135-148.

393 Lima-Rosa, C. A., C. W. Canal, P. R. Vargas Fallavena, L. B. de Freitas, and F. M. Salzano.
394 2005. LEI0258 microsatellite variability and its relationship to B-F haplotypes in
395 Brazilian (blue-egg Caipira) chickens. Genet. Mol. Biol. 28:386–389.

396 Livant, E. J., and S. J. Ewald. 2005. High-resolution typing for chicken BF2 (MHC class I)
397 alleles by automated sequencing. Anim. Genet. 36:432–434.

398 Lwelamira, J., G. C. Kifaro, P. S. Gwakisa, and P. L. M. Msoffe. 2008a. Association of
399 LEI0258 microsatellite alleles with antibody response against Newcastle disease virus

vaccine and body weight in two Tanzania chicken ecotypes. African J. Biotechnol. 7:714–720.

Manjula, P., B. Bed'Hom, M. R. Hoque, S. Cho, D. Seo, O. Chazara, S. H. Lee, and J. H. Lee. 2020a. Genetic diversity of MHC-B in 12 chicken populations in Korea revealed by single-nucleotide polymorphisms. Immunogenetics 72:367–379.

Manjula, P., J. E. Fulton, Dongwon-Seo, and J. H. Lee. 2020b. Major Histocompatibility Complex B (MHC-B) Variability in Korean Native Chicken. Poult. Sci. 99:4704-4713.

Miller, M. M. and R. L. Taylor. 2016. Brief review of the chicken Major Histocompatibility Complex: The genes, their distribution on chromosome 16, and their contributions to disease resistance. Poult. Sci. 95:375–392.

Mpenda, F. N., C. K. Tiambo, M. Kyallo, J. Juma, R. Pelle, S. L. Lyantagaye, and J. Buza. 2020. Association of LEI0258 Marker Alleles and Susceptibility to Virulent Newcastle Disease Virus Infection in Kuroiler, Sasso, and Local Tanzanian Chicken Embryos. *Journal of Pathogens*, vol. 2020, Article ID 5187578, 8 pages, 2020. https://doi.org/10.1155/2020/5187578

Mwambene, P. L., M. Kyallo, E. Machuka, D. Githae, and R. Pelle. 2019. Genetic diversity of 10 indigenous chicken ecotypes from Southern Highlands of Tanzania based on Major Histocompatibility Complex-linked microsatellite LEI0258 marker typing. Poult. Sci. 98:2734–2746.

Nguyen-Phuc, H., J. E. Fulton, and M. E. Berres. 2016. Genetic variation of major histocompatibility complex (MHC) in wild Red Junglefowl (Gallus gallus). Poult. Sci. 95:400–411.

Nikbakht, G., A. Esmailnejad, and N. Barjesteh. 2013. LEI0258 microsatellite variability in Khorasan, Marandi, and Arian chickens. Biochem. Genet. 51:341–349.

Nikbakht, G., and A. Esmailnejad. 2015. Chicken major histocompatibility complex polymorphism and its association with production traits. Immunogenetics 67:247–252.

Owen, J.P., M.E. Delany, and B. A. Mullens. 2008. MHC haplotype involvement in avian resistance to an ectoparasite. Immunogenet. 60:621-31.

Raymond, M., and F. Rousset. 1995. An Exact Test for Population Differentiation. Evolution Vol. 49, No. 6, pp. 1280-1283. DOI: 10.2307/2410454. https://www.jstor.org/stable/2410454

Revidatti, F., J. F Rafart, J. C Terraes, R. J. Fernandez, G. L. Sandoval, M. V. Asiain, and M. M Sindik. 2005. Reproductive output in laying hen and meat type breed crosses. InVet 2005, 7(1), 19-23[10 de March, 2021]. ISSN: 1514-6634. Retrieved from https://www.redalyc.org/articulo.oa?id=179114156002

Robertson, A., and W. G. Hill. 1984. Deviations from Hardy-Weinberg proportions: Sampling variances and use in estimation of inbreeding coefficients. Genetics 107:703–718.

Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Resources 8: 103-106.

Schou, T. W., R. Labouriau, A. Permin, J. P. Christensen, P. Sorensen, H. P. Cu, V. K. Nguyen, and H.R. Juul-Madsen. 2010. MHC haplotype and susceptibility to experimental infections (*Salmonella enteritidis, Pasteurella multocida or Ascaridia galli*) in a commercial and an indigenous chicken breed. Vet. Immunol. Immunopath. 135:52-63.

Semagn, K., R. Babu, S. Hearne, and M. Olsen. 2014. Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): Overview of the technology and its application in crop improvement. Mol. Breed. 33:1–14.

Tarrant, K. J., R. Lopez, M. Loper, and J. E. Fulton. 2020. Assessing MHC-B diversity in Silkie chickens. Poult. Sci. 99:2337–2341.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-Statistics for the Analysis of Population Structure. Evolution (N. Y). 38:1358

FIG 1

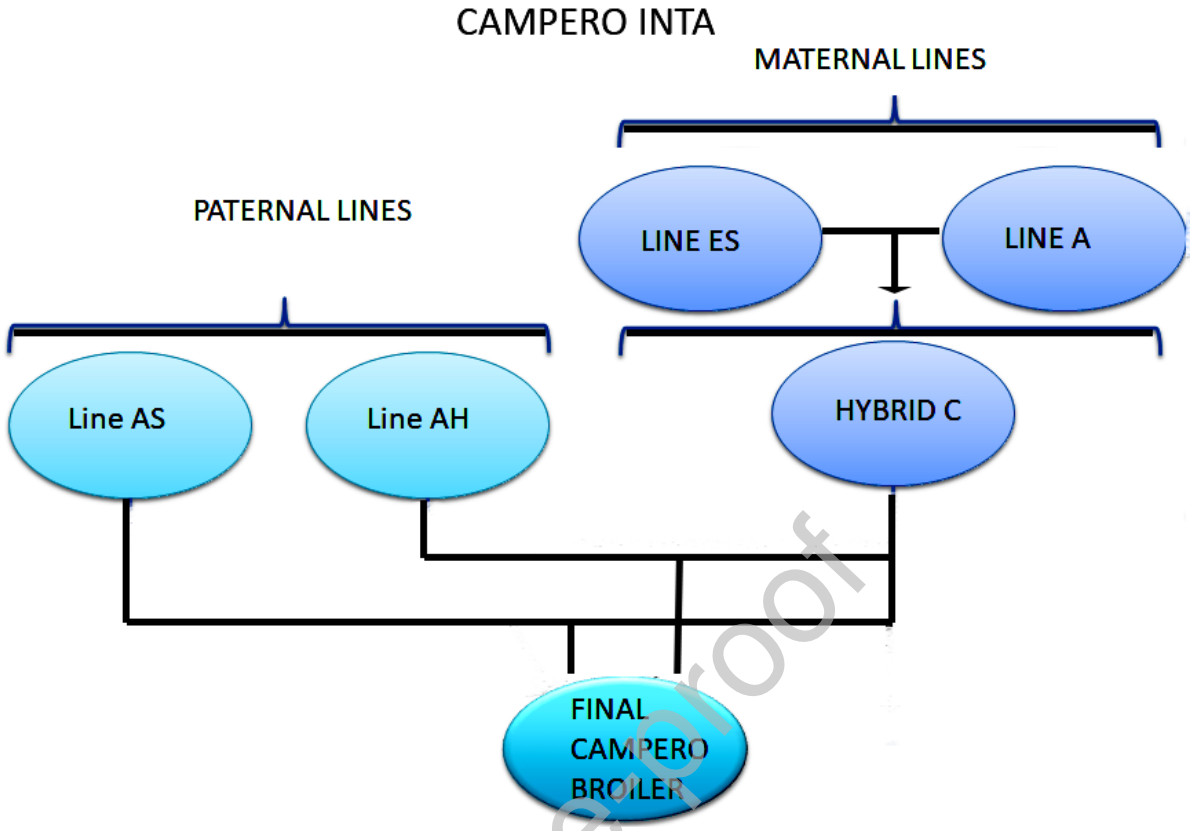

Figure 2

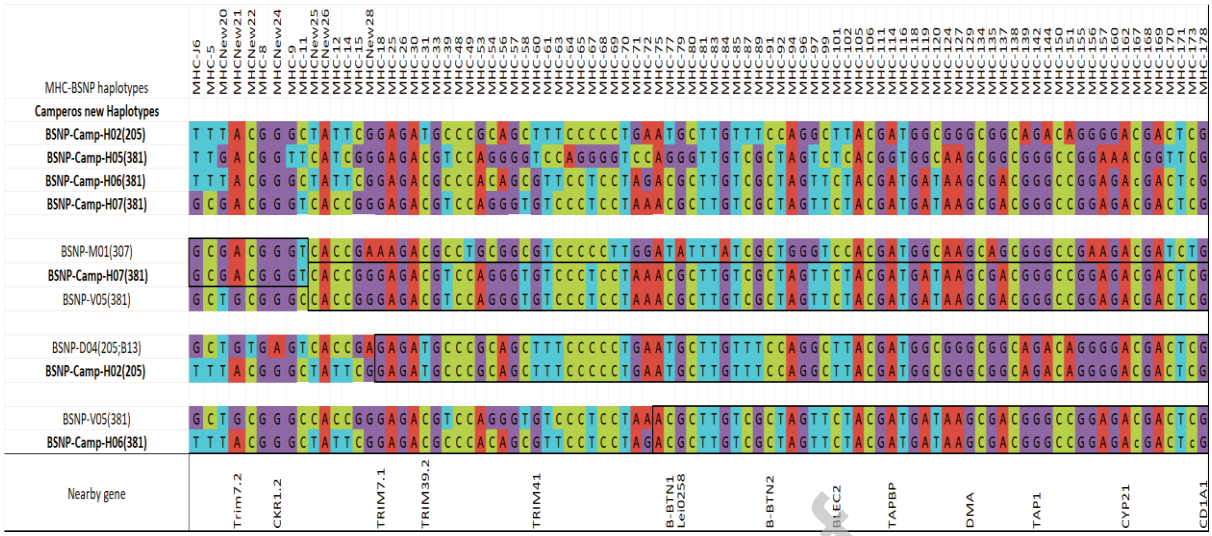

Table 1. MHC-BSNP haplotypes with LEI0258 allele size and their frequencies in the four parental lines utilized to produce the hybrid Campero chicken

BSNP Haplotype	LEI0258 size	Breed*	Line A	Line AS	Line ES	Line AH (2018) [#]
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

* these haplotypes were previously reported in specific breeds
BRL=broiler; RJF=Red Jungle fowl; NH=New Hampshire; RIR=Rhode Island Red; WPR=White Plymouth Rock: WL=White Leghorn
[#] from Iglesias et al., 2019

Conflict of interests

All the author declare that the 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