Mhc-B haplotypes in "Campero-Inta" chicken synthetic line

Gabriela M. Iglesias ,[*,1] Zulma E. Canet,[†,‡] Horacio Cantaro,[§,#] María C. Miquel,[‖,2] Julián E. Melo,[**,††] Marcia M. Miller,[‡‡] Mark E. Berres,[§§] and Janet E. Fulton[§§,##]

[*] *Universidad Nacional de Río Negro, Sede Alto Valle y Valle Medio, Escuela de Veterinaria y Producción Agroindustrial, Area de Genética, Choele Choel, Rio Negro 8360, Argentina;* [†] *Cátedra de Genética, Facultad de Ciencias Veterinarias, Universidad Nacional de Rosario, Boulevard Ovidio Lagos y Ruta 33, Casilda, Santa Fe 2170, Argentina;* [‡] *INTA Pergamino, Estación Experimental Agropecuaria "Ing. Agr. Walter Kugler", Pergamino, Buenos Aires 2700, Argentina;* [§] *Universidad Nacional de Río Negro, Sede Alto Valle y Valle Medio, Escuela de Veterinaria y Producción Agroindustrial, Area de Producción Aves y Pilíferos, Choele Choel, Rio Negro 8360, Argentina;* [#] *INTA, Proyecto Nacional de Avicultura (PAVI), Estación Experimental Agropecuaria Alto Valle, Programa Nacional de Producción Animal, Ruta Nacional 22, Argentina;* [‖] *Cátedra de Genética, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Buenos Aires 8332, Argentina;* [**] *Facultad de Ciencias Agrícolas, Universidad Católica Pontificia Argentina (UCA), Buenos Aires, C.A.B.A 1107, Argentina;* [††] *Departamento de Tecnología, Universidad Nacional de Luján (UNLu), B6702 Luján, Buenos Aires, Argentina;* [‡‡] *Department of Molecular and Cellular Biology, Beckman Research Institute of the City of Hope, Duarte, CA 91010–3000;* [§§] *Biotechnology Center, University of Wisconsin, Madison, WI 53706; and* [##] *Hy-Line International, Dallas Center, IA 50063*

ABSTRACT The major histocompatibility complex-*B* (MHC-*B*) in chickens is a cluster of genes located on chromosome 16. The chicken MHC-*B* is known to be highly associated with resistance to numerous diseases caused by viruses, bacteria, and parasitic pathogens. Since the level of resistance varies with MHC-*B* haplotypes, identification and classification of different haplotypes within lines is important for sustaining lines. The "Campero-INTA" chicken breed is a meat-type free-range poultry breed that was developed specifically for small producers in Argentina. Campero-INTA was started by selection in populations produced by crosses between a variety of established lines. MHC-*B* variation was examined in 65 samples obtained in 2002 using the VNTR marker LEI0258, a marker for MHC-*B* region. These samples plus and an additional 55 samples from 2018 were examined for variation using the MHC-*B* specific SNP panel that encompasses ∼230,000 bp of the MHC-*B* region. Eleven MHC-*B* SNP haplotypes with 6 LEI0258 alleles were identified in the 120 samples representing the Campero-INTA AH (male) line. Seven haplotypes originate from the breeds originally used in the development of Campero-INTA AH line. Two appear to be recombinant haplotypes. The origin of the remaining 2 is not known, but may be associated with genes introduced from crosses with the Fayoumi breed conducted more recently to sustain the line.

Key words: MHC-*B*, chickens, Campero-INTA, haplotypes, SNPs, LEI0258, Fayoumi

2019 Poultry Science 0:1–6
http://dx.doi.org/10.3382/ps/pez431

INTRODUCTION

The major histocompatibility complex-*B* (MHC-*B*) in chickens is a cluster of genes located on chromosome 16. This gene complex was originally identified as the B blood group. A total of 27 different haplotypes (B1-B29) were defined using alloantisera (Briles and Briles, 1982). The detection of variation within the chicken MHC-*B* is of great interest as this variation has been shown to be highly associated with resistance to diseases caused by viral, bacterial, and parasitic pathogens (Hansen et al., 1967; Briles et al., 1977; Collins et al., 1979; Lamont et al., 1987; Lillehoj et al., 1988; Cotter et al., 1998; Taylor, 2004; Schou et al., 2007; Owen et al., 2008; Goto et al. 2009; Lwelamira et al., 2009).

The production and use of alloantisera for detection of MHC-*B* types works well for lines with limited MHC-*B* variability, such as inbred and MHC-*B* defined lines. Most early MHC-*B* work was done using the White Leghorn breed (Briles and Briles, 1982). Alloantisera are consumable biological reagents. It can be difficult to consistently reproduce alloantisera with the same

© 2019 Poultry Science Association Inc.
Received March 20, 2019.
Accepted July 12, 2019.
[1]Corresponding author: giglesias@unrn.edu.ar
[2]Deceased.
This work was founded by the following grants: UNRN 40-A-498 2016, INTA Grant Number PNCAR333 2005 and PICT 2006 01383.

Downloaded from https://academic.oup.com/ps/advance-article-abstract/doi/10.3382/ps/pez431/5543517 by EKU Libraries user on 04 August 2019

target specificity. Antisera can be cross-reactive between different MHC-*B* types (Fulton et al., 2001) leading to ambiguous identification. Serotyping has not worked very well to identify MHC-*B* haplotypes in breeds other than White Leghorn and in meat type chickens. Until recently, not much was known about the MHC-*B* diversity and relative disease resistance in most breeds of chickens.

Several DNA-based techniques have been used to examine MHC-*B* diversity in chickens. Southern blots using MHC-*B* gene-specific probes have revealed much MHC-*B* diversity in many chicken breeds (Andersson et al., 1987; Miller et al., 1988; Chausse et al., 1989; Uni et al., 1995; Li et al., 1997; Yonash et al., 1999; Melo et al., 2002; Iglesias et al., 2003; Lima-Rosa et al., 2005). Single-strand conformation polymorphism has been utilized to examine small regions of specific genes (Goto et al., 2002). Direct sequencing of specific genes has also been used to identify haplotypes (Livant et al., 2001; Goto et al., 2002; Lima-Rosa et al., 2005; Iglesias et al., 2007; Worley et al., 2008). These techniques were particularly useful to examine MHC variation in chickens found in different breeds. All these techniques are not suited for screening large number of animals.

The microsatellite LEI0258 is a VNTR (variable number of tandem repeat) region located within the MHC-*B*. LEI0258 contains tandem repeats of 12 and 13 bp plus several indels in the flanking region that result in the size differences that differentiate alleles (McConnell et al., 1999; Fulton et al., 2006). Due to the large size of the 2 tandem repeats, allelic size variation is large. Alleles can be easily distinguished using electrophoresis to separate PCR products in agarose gels. LEI0258 has been used to identify MHC-*B* variation in Leghorn lines, brown egg layers, and indigenous types of chickens from multiple countries including Africa, Iran, Korea, and India (Lima-Rosa et al., 2005; Fulton et al., 2006, 2013; Chazara et al., 2013; Han et al., 2013; Nikbakht et al., 2013). In a survey of 80 populations from multiple countries, 79 LEI0258 alleles were detected (Chazara et al., 2013). There are limitations to MHC-*B* typing with LEI0258. Different MHC-*B* haplotypes can have the same LEI0258 allele size. Mutation can result in different LEI0258 allele sizes being found within the same MHC- *B* haplotypes (Fulton et al., 2006, 2016b). In addition, differences in allele size estimates made in different labs can occur and confound the clarity of type assignments. Since LEI0258 is a single marker, only one location within the MHC is evaluated.

More recently, SNP typing for MHC-*B* has been developed. A panel of SNPs encompassing 210,000 bp of the chicken MHC-*B* region was initially developed by Chazara et al. (2010). This panel was subsequently modified and expanded to include an additional upstream *BG* gene and validated in several genotyping tests with multiple chicken lines, heritage breeds, and wild Jungle Fowl (Fulton et al., 2016a,b, 2017; Nguyen-Phuc et al., 2016). The SNP panel, which includes LEI0258 types, has made it easy to distinguish haplotypes, identify novel recombinants, and define MHC variation in chickens from multiple sources.

The Campero synthetic line of chickens was developed by INTA (*Instituto Nacional de Tecnología Agropecuaria*) in the 1980s in an experimental station located in Pergamino, Buenos Aires, Argentina. It was derived from crosses of standard breeds to provide a slower growing poultry that could perform well on pasture without significant feed input and was well adapted to local conditions in Argentina. The primary purpose was to provide an improved breed for small-scale producers of free-range poultry. Standard breeds used to develop the Campero breed. These included Cornish, Rhode Island Red, Barred Plymouth Rock, and New Hampshire. From a core population, parental lines were developed. This included sire lines "AH" and "AS" and dam lines "A" and "ES" (Canet et al., 2011, Dottavio and Di Masso, 2011). These lines were first selected for specific phenotypic characteristics and then randommated for more than 20 generations.

The production "Campero" bird is hybrid produced by a cross of a one of the male synthetic lines (either "AH" or "AS") with a female hybrid (produced by a single cross of "ES" and "A").

The Fayoumi breed was introgressed in 2002 to increase population size and reduce consanguinity. Also beginning in 2002, a selection program was utilized for 7 generations, using feed intake (**FI**) and live weight at 54 to 75 D to improve these 2 performance parameters. (Melo et al., 2006, 2010). Here we present MHC-*B* haplotype information obtained from the Campero male line AH.

MATERIALS AND METHODS

Samples

The male parent synthetic line "AH" from INTA Pergamino (Buenos Aires, Argentina) was sampled 2 different times in 2002 and 2018. The core of the line was composed of 120 females and 12 males in 2002 and 45 females and 5 males in 2018. The first sample set of 85 males was obtained in 2002 with the second set of 55 birds collected in 2018 (males and females). Blood samples were obtained from the wing vein, and DNA was isolated either from 200 μL of blood (frozen or fresh) by salt/ethanol precipitation (2002 samples) or from dried blood spot on FTA Elute cards (GE Healthcare) (2018 samples). DNA was extracted from the FTA Elute card following the manufacturer's recommendations.

LEI0258 Microsatellite Analysis

Amplification of LEI0258 locus was performed using primers proposed by McConnell et al. (1999) (Forward: 5′-CACGCAGCAGAACTTGGTAAGG-3′ and Reverse: 5′-AGCTGTGCTCAGTCCTCAGTGC-3′) with the forward primer being dye-labelled with 6-Fam

Downloaded from https://academic.oup.com/ps/advance-article-abstract/doi/10.3382/ps/pez431/5543517 by EKU Libraries user on 04 August 2019

Table 1. A total of 11 MHC-BSNP haplotypes (with LEI0258 allele size) found in 2002 (n = 85) and 2018 (n = 55) samples of Campero-INTA sire line and their frequencies.

Haplotype	2002	2018
BSNP-A08(357)	0.05	
BSNP-A09(357)	0.09	
BSNP-D04(205)	0.01	0.01
BSNP-K03(261)	0.05	
BSNP-M01(307)	0.16	0.05
BSNP-Q01(193)	0.42	0.42
BSNP-V05(381)	0.16	0.35
BSNP-Camp-Hap01(205)	0.01	
BSNP-Camp-Hap02(205)	0.05	
BSNP-Camp-Hap03(381)	0.01	
BSNP-Camp-Hap04[1]		0.17

[1]LEI0258 allele size was not determined for BSNP-Camp-Hap04.

as described by Fulton et al. (2006). Allele sizes were estimated following electrophoresis on a CEQ8800 capillary sequencer (Beckman-Coulter, Fullerton, CA) and analysis using CEQ 8800 fragment analysis software. LEI0258 information was obtained for a subset of samples that included at least one representative of each haplotype for the first set of samples only.

MHC-B SNP Typing

The SNP genotyping of the MHC region was done using a high-density SNP panel, as described by Fulton and colleagues in 2016. The SNP panel consisted of 101 SNPs, encompassing 230,000 bp of the chicken MHC. This covers 45 genes of the MHC-*B* region, including *BG2* through *CD1A1*. The genotyping chemistry utilizes competitive allele specific PCR primers (KASP) as described by Semagn et al. (2013). Primer sequence information used for each of the SNP KASP assays is provided within Fulton et al. (2016b). Genotypes and haplotype information for the study herein was derived from 90 SNPs in the Fulton et al. (2016b) panel (Table 1). Eleven SNPs that hybridize in the repetitive *BG* region were excluded. Exact location of those SNP can be found in Fulton et al. (2016b). Genes nearby to some SNPs are shown at the bottom of the table to assist in orientation of the SNP within the MHC region.

Analysis of MHC-B Haplotype Variation

Observed and expected heterozygosity (the latter referring to Nei's unbiased gene diversity) of both populations was calculated with hierfstat v0.04–28 (Nei, 1973) (Goudet and Jombart, 2015). Next, we tested the hypothesis that haplotype frequencies followed the Hardy–Weinberg equilibrium. A population-level chi-square statistic was computed for LEI0258 alleles, and significance was tested with a Monte Carlo permutation procedure consisting of 1,000 replicates (hw.test; Paradis, 2010). The analysis of molecular variance (**AMOVA**) procedure (Excoffier et al., 1992) implemented in Poppr v2.7.1 was used to partition the proportion of total genetic variation explained among populations and within individuals. Significance was tested with 1,000 randomized permutations using the function randtest in the R package ade4 (Dray and Dufour, 2007).

RESULTS AND DISCUSSION

MHC Diversity

MHC-*B* SNP panel genotyping identified 10 haplotypes within the Campero AH synthetic line in 2002. Testing of the smaller sample set in 2018 sampling found only 5 haplotypes (Table 1), one of which was not the 2002 sample set. Overall, 11 different haplotypes were found. A subset of the 2002 samples was tested for LEI0258 alleles ensuring that at least one representative of each MHC-BSNP haplotype was included. Figure 1 lists the specific SNP allelic combination which defines each haplotype. Information from both the earlier LEI0258 typing and the MHC-*B* SNP panel, which also includes LEI0258, is combined and included within the haplotype name assigned. Each MHC-*B* haplotype found in the Campero AH synthetic line was compared with those previously reported in standard breeds, heritage broilers, MHC-defined lines, and commercially utilized breeds as reported by Fulton et al. (2016a). Seven of the haplotypes are identical to those found previously, including the LEI0258 allele. Some of these are identical to haplotypes found in the White Plymouth Rock (BSNP-A08, BSNP-M01) and Rhode Island Red breeds (BSNP-A08, BSNP-M01). Other haplotypes are identical to haplotypes in heritage broiler populations (BSNP-A09, BSNP-D04, BSNP-K03, BSNP-Q01, and BSNP-V05). Two of the haplotypes (BSNP-D04 and BSNP-K03) are also present in the White Leghorn breed (Fulton et al., 2016a,b). Finding these haplotypes in the Campero-INTA synthetic line reflects the contributions of the lines used originally in developing the Camperos.

Four novel haplotypes have been found in the Camperos AH line. These have been assigned the names BSNP-Camp-Hap01 through Hap04. Two appear to be novel haplotypes not identified in the original stock from which they are derived. The other 2 appear to be recombinant haplotypes that are the result of crossing over events (Figure 1). In BSNP-Camp-H03, crossover appears to have occurred between 2 haplotypes within the line, BSNP-Q01 and BNSP-V05. The breakpoint is somewhere between SNP MHC-15 (59,015 bp) and SNP MHC-39 (89,076 bp) which overlaps the most frequent MHC-B recombination hotspot identified by Fulton et al. (2016b). The second apparent recombinant is BSNP-Camp-H02. A parental haplotype for the beginning section of this haplotype was not identified. The downstream portion, beginning at SNP MHCNew28 (62,274 bp), shows identity with BSNP-D04. The occurrence of MHC recombinants is not unexpected. MHC-*B* recombinant haplotypes have been detected previously with this SNP panel with an estimated rate of 7 per 2,500 meioses (Fulton et al. 2016a,b).

Downloaded from https://academic.oup.com/ps/advance-article-abstract/doi/10.3382/ps/pez431/5543517 by EKU Libraries user on 04 August 2019

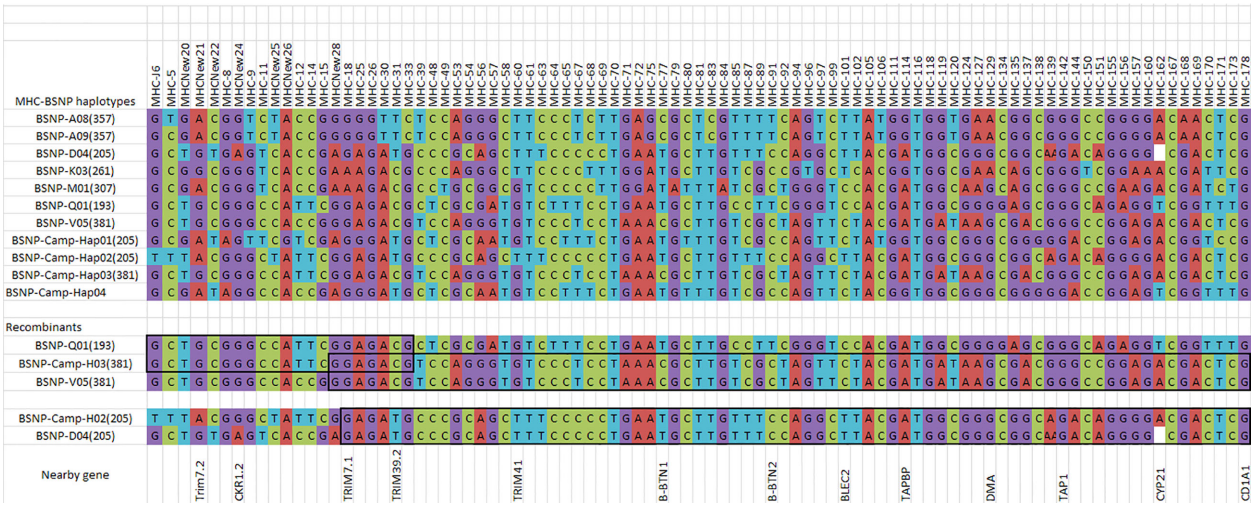

Figure 1. BSNP haplotypes found in the AH line (paternal line of Campero Breed). Two new putative recombinants haplotypes are noted. A portion of the genes within the region covered by the SNP panel are noted. This figure will also be sent in a separated file for better visualization.

In the first sample of 85 birds collected in 2002, we could not reject the null hypothesis of the Hardy–Weinberg equilibrium among the 11 MHC-*B* haplotypes identified (Ho = 0.741, He = 0.747, $P = 0.652$). However, haplotype frequencies in the second sample of 55 birds collected in 2018 were not in expected Hardy–Weinberg proportions (Ho = 0.872, He = 0.691, $P = 0.0013$). AMOVA indicated statistically significant evidence for a temporal change in MHC-*B* haplotype frequencies. The majority of variation (69.35%) occurred within individuals, while 3.95% was explained by variation among sampling times ($F_{ST} = 0.0395$, $P < 0.0001$). The introgression of Fayoumi genetics that occurred in 2002 appears to have introduced an additional novel haplotype into the synthetic paternal line (AH). The Fayoumi breed was originally developed in Egypt and is reported to be a more disease-resistant breed (Pinard-Van Der Laan et al., 1998; Saelao et al., 2018).

The VNTR marker LEI0258 has been commonly used as an indicator of MHC diversity in multiple indigenous populations (Lima-Rosa et al., 2005; Hoque et al., 2011; Izadi et al., 2011; Chazara et al., 2013; Han et al., 2013; Nikbakht et al., 2013; Ncube et al., 2014; Touko et al., 2015), due to its low cost and ease of use. Fulton et al., 2006) described the limitations of this marker, which included the occurrence of multiple size alleles for the same MHC-*B* haplotype and a mutation rate of the internal repeats. These limitations can lead to either an underestimation or overestimation of the number of MHC haplotypes present. Furthermore, since LEI0258 is a single point within the MHC-*B*, it will not indicate recombinant haplotypes. This Campero population data set shows that LEI0258 underestimates the MHC haplotype variation as the 10 haplotypes within the 2002 sampling contained only 6 unique LEI0258 allele sizes. It failed to distinguish between the 2 haplotypes BSNP-A08 and BNSP-A09, and neither did it indicate the presence of the MHC recombinants nor distinguish them from other haplotypes. These limitations of the sole use LEI0258 as an indicator of MHC-*B* variability can clearly be seen within this Campero population data set.

We report here the LEI0258 allele sizes as estimated size separation following electrophoresis. Additional variation is likely to be present within the LEI0258 allele due to small deletions/insertions or single base changes. This additional variation within LEI0258 has been reported for multiple breeds (Fulton et al., 2006; Chazara et al., 2013; Han et al., 2013; Guangxin et al., 2014). It would be interesting to sequence multiple examples of the LEI0258 alleles found in the Campero synthetic AH line to determine what additional level of variation might exist in these alleles.

The dataset presented here captures MHC diversity from 2 different generations, separated by 16 yr. The number of unique haplotypes found decreased from 10 in 2002 to only 5 in 2018. Although most haplotype variation was found within populations, approximately 4% was attributed to haplotype differentiation over a short period of time, perhaps due to significant introgression of the Fayoumi breed during the flock improvement phase. This is of some concern as it suggests that MHC diversity has been lost over the intervening time. However, those MHC haplotypes that were not detected in the 2018 sampling were at lower frequency in the earlier sampling, with some of them being detected in only 1–2 individuals. Sample size would be critical in determining if these MHC haplotypes are still present within the line. A more complete sampling of the line would be a better indicator of the continued presence of specific MHC types.

The presence of novel MHC-BSNP haplotypes within the Campero AH synthetic line is not totally unexpected. Within the Finnish Landrace breed, 20 out of the 36 haplotypes present were different from those

Downloaded from https://academic.oup.com/ps/advance-article-abstract/doi/10.3382/ps/pez431/5543517 by EKU Libraries user on 04 August 2019

previously found by Fulton et al. (2016a) and Fulton (2017), and a survey of German Traditional and US rare Breeds showed that close to 50% of the haplotypes found within a breed were novel (Fulton et al., 2017).

Close examination of the Campero synthetic AH line haplotypes information (Table 1) reveals that a small subset of the 90 SNPs used can identify the 11 possible haplotypes. For example, BSNP-K03 is unique at SNP MHC-150, having the T allele whereas all other haplotypes found in this line have the C allele. A small subset of 6–8 SNPs could be used to routinely determine MHC type within this population, rather than genotyping all of the SNP. This would allow studies on MHC associations with disease traits and other performance impacts. While this study utilized KASP chemistry single plex assays, any other method of SNP identification can be utilized to determine genotype.

The prime function of the Campero synthetic line hybrid is to provide a hardy, free-range bird that is well adapted to local Argentinian small-holder conditions. Due to the known strong associations between MHC and disease resistance, better understanding of the MHC variation present within this synthetic line, and any associations with disease resistance will help to focus selection for improved performance under natural disease challenges. This can improve the health and well-being of this synthetic line in its natural environment. The knowledge of the haplotypes of MHC in these populations acquires greater importance in these animals as they are reared with minimal prophylaxis and hence natural resistance to diseases is very important.

Further research on the Campero synthetic lines should examine the MHC diversity in the other sire line (AS) as well as both of the dam lines (A and ES) used to produce the final production bird. Disease studies looking for increased resistance to specific disease challenges associated with specific MHC haplotypes would allow for focused selection for improved disease resistance.

ACKNOWLEDGMENTS

Thanks to Miguel Javier Huguet for extraction of DNA samples from (from FCV, University of Buenos Aires. Argentina), and the Hy-Line Molecular Genetics personnel for DNA extractions from FTA cards and for genotyping.

REFERENCES

Andersson, L., C. Lundberg, L. Rask, B. Birgitte-Nielsen, and M. Simonsen. 1987. Analysis of class II genes of the chicken MHC (B) by use of human DNA probes. Immunogenetics 26:79–84.

Briles, W. E., and R. W. Briles. 1982. Identification of haplotypes of the chicken major histocompatibility complex (*B*). Immunogenetics 15:449–459.

Briles, W. E., H. A. Stone, and R. K. Cole. 1977. Marek's disease: effects of B histocompatibility alloalleles in resistant and susceptible chicken lines. Science 165:193–195.

Canet, Z. E., A. M. Dottavio, M. V. Fain Binda, B. M. Romera, and R. J. Di Masso. 2011. Body condition at slaughter of hens from five maternal strains used as breeders for the production of free-range broilers. XXII Latin American Poultry Congress 2011. Engormix. Retrieved 26 October 2016, 1–10. http://en.engormix.com/MA-poultry-industry/genetic/articles/body-condition-slaughter-hens-t1825/103-p0.htm).

Chausse, A. M., F. Coudert, G. Dambrine, F. Guillemot, M. M. Miller, and C. Auffray. 1989. Molecular genotyping of four chicken B-complex haplotypes with B-Lβ, B-F, and B-G probes. Immunogenetics 29:127–130.

Chazara, O., C. S. Chang, N. Bruneau, K. Benabdeljelil, J. C. Fotsa, B. B. Kayang, N. E. Loukou, R. Osei-Amponsah, V. Yapi-Gnaore, I. A. Youssao, C. F. Chen, M. H. Pinard-van der Laan, M. Tixier-Boichard, and B. Bed'hom 2013. Diversity and evolution of the highly polymorphic tandem repeat LEI0258 in the chicken MHC-*B* region. Immunogenetics 65:447–459.

Chazara, O., J. Fulton, H. Juul-Madsen, C. S. Chang, and B. Bed'Hom. 2010. High-resolution chicken MHC genotyping using a SNP panel. Proceedings of the 32nd Conference of the International Society for Animal Genetics, July 26–30th, Edinburgh, UK, 138.

Collins, W. M., W. E. Briles, A. C. Corbett, K. K. Clark, R. M. Zsigray, and W. R. Dunlop. 1979. *B* locus (MHC) effect upon regression of RSV-induced tumors in noninbred chickens. Immunogenetics 9:97–100.

Cotter, P. F., R. L. Taylor, Jr., and H. Abplanalp. 1998. B-complex associated immunity to Salmonella enteritidis challenge in congenic chickens. Poult. Sci. 77:1846–1851.

Dottavio, A. M., and R. J. Di Masso. 2011. Mejoramiento avícola para sistemas productivos semi-intensivos que preservan el bienestar animal. J. Basic Appl. Genet. 21:1–10.

Dray, S., and A. Dufour, 2007. The ade4 package: implementing the duality diagram for ecologists. J. Stat. Soft. 22:1–20.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Fulton, J. E. 2017. MHC-B Diversity of Domestic Chickens. Xth European Symposium on Poultry Genetics, WPSA Proceedings, Ploufragan, France, 19-22 June 2017.

Fulton, J. E., J. Arango, J. A. Arthur, P. Settar, K. S. Kreager, and N. P. O'Sullivan. 2013. Improving the outcome of a Marek's disease challenge in multiple lines of egg type chickens. Avian Dis. 57(No. 2 Suppl):395–400.

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

Fulton, J. E., H. D. Hunt, and L. D. Bacon. 2001. Chicken major histocompatibility complex class I definition using antisera induced by cloned class I sequences. Poult. Sci. 80:1554–1561.

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

Fulton, J. E., A. R. Lund, A. M. McCarron, K. N. Pinegar, D. R. Korver, H. L. Classen, and M. E. Berres. 2016a. MHC variability in heritage breeds of chickens. Poult. Sci. 95:393–399.

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

Goto, R. M., M. Afanassieff, J. Ha, G. M. Iglesias, S. J. Ewald, W. E. Briles, and M. M. Miller. 2002. Single-strand conformation polymorphism (SSCP) assays for major histocompatibility complex B genotyping in chickens. Poult. Sci. 81:1832–1841.

Goto, R. M., Y. Wang, R. L. Taylor, Jr., P. S. Wakenell, K. Hosomichi, T. Shiina, C. S. Blackmore, W. E. Briles, and M. M. Miller. 2009. BG1 has a major role in MHC-linked resistance to malignant lymphoma in the chicken. Proc. Natl. Acad. Sci. USA 106: 16740–16745.

Downloaded from https://academic.oup.com/ps/advance-article-abstract/doi/10.3382/ps/pez431/5543517 by EKU Libraries user on 04 August 2019

Goudet, J., and T. Jombart. 2015. R Package 'hierfstat'. December 4, 2015. Version 0.04-22. Retrieved from: http://www.r-project.org, http://github.com/jgx65/hierfstat.

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

Han, B., L. Lian, L. Qu, J. Zheng, and N. Yang. 2013. Abundant polymorphisms at the microsatellite locus LEI0258 in indigenous chickens. Poult. Sci. 92:3113–3119.

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

Hoque, M. R., S. H. Lee, K. C. Jung, B. S. Kang, M. N. Park, H. K. Lim, K. D. Choi, and J. H. Lee. 2011. Discrimination of Korean Native Chicken populations using SNPs from mtDNA and MHC polymorphisms. Asian Australas. J. Anim. Sci. 24:1637–1643.

Iglesias, G. M., M. J. Huguet, R. M. Goto, M. C. Miquel, and M. M. Miller. 2007. Report of new alleles at *BG* loci in Campero chickens. J. Basic Appl. Genet. 18:29–30.

Iglesias, G. M., L. A. Soria, R. M. Goto, A. M. Jar, M. C. Miquel, O. J. Lopez, and M. M. Miller. 2003. Genotypic variability at the major histocompatibility complex (B and Rfp-Y) in Camperos broiler chickens. Anim. Genet. 34:88–95.

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

Laan, P.-V. D., J. L. Monvoisin, P. Pery, N. Hamet, and M. Thomas. 1998. Comparison of outbred lines of chickens for resistance to experimental infection with coccidiosis (Eimeria tenella). Poult. Sci. 7:185–191.

Lamont, S. J., C. Bolin, and N. Cheville. 1987. Genetic resistance to fowl cholera is linked to the major histocompatibility complex. Immunogenetics 25:284–289.

Li, L., W. L. Johnson, and S. J. Ewald. 1997. Molecular characterization of major histocompatibility complex (B) haplotypes in broiler chickens. Anim. Genet 28:258–267.

Lillehoj, H. S. 1988. Influence of inoculation dose, inoculation schedule, chicken age, and host genetics on disease susceptibility and development of resistance to *Eimeria tenella* infection. Avian Dis. 32:437–444.

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

Livant, E. J., D. Zheng, L. W. Johnson, W. Shi, and S. J. Ewald. 2001. Three new MHC haplotypes in broiler breeder chickens. Anim. Genet. 32:123–131.

Lwelamira, J., G. C. Kifaro, and P. S. Gwakisa. 2009. Genetic parameters for body weights, egg traits and antibody response against Newcastle Disease Virus (NDV) vaccine among two Tanzania chicken ecotypes. Trop. Anim. Health Prod. 41:51–59.

McConnell, S. K. J., D. A. Dawson, A. Wardle, and T. Burke. 1999. The isolation and mapping of 19 tetranucleotide microsatellite markers in the chicken. Anim. Genet. 30:183–189.

Melo, J. E. 2010. Objetivo de selección para la producción del pollo Campero-INTA Phd Thesis. Facultad de Ciencias Veterinarias. Universidad de Buenos Aires. Argentina.

Melo, J. E., G. M. Iglesias, L. A. Soria, G. Mallo, M. J. Huguet, Z. E. Canet, and M. C. Miquel. 2002. Selection criteria for free range broilers including quantitative traits and molecular markers. Page 87. Proc. VII World Congr. on Genetics Applied to Livestock Prod., Montpellier, France.

Melo, J. E., E. Romano, Z. E. Canet, and M. C. Miquel. 2006. Genetic parameters of growth and feed efficiency in a free-range broiler stock. Proceedings of the 8th World Congress on Genetics Applied to Livestock Production 13–18. Belo Horizonte, Brazil.

Miller, M. M., H. Abplanalp, and R. Goto. 1988. Genotyping chickens for the B-G subregion of the major histocompatibility complex using restriction fragment length polymorphisms. Immunogenetics 28:374–379.

Ncube, K. T., P. J. Jooste, P. Soma, E. F. Dzomba, and F. C. Muchadeyi. 2014. Polymorphism of the major histocompatibility complex and genetic structure of southern african village chicken populations. Int. J. Poult. Sci. 13:357–363.

Nei, M. Analysis of gene diversity in subdivided populations. 1973. Proc. Natl. Acad. Sci. USA 70:3321–3323.

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.

Owen, J. P., M. E. Delany, and B. A. Mullens. 2008. MHC haplotype involvement in avian resistance to an ectoparasite. Immunogenetics 60:621–631.

Paradis, E. 2010. pegas: An R package for population genetics with an integrated-modular approach. Bioinformatics 26:419–420.

Saelao, P., Y. Wang, R. Gallardo, S. J. Lamont, J. M. Dekkers, T. Kelly, and H. Zhou. 2018. Novel insights into the host immune response of chicken Harderian gland tissue during Newcastle disease virus infection and heat treatment. BMC Vet. Res. 14: 280.

Schou, T. W., A. Permin, H. R. Juul-Madsen, P. Sorenson, R. Labouriau, T. L. H. Nguyen, M. Fink, and S. L. Pham. 2007. Gastrointestinal helminths in indigenous and exotic chickens in Vietnam: association of the intensity of infection with the major histocompatibility complex. Parasitology 134:561–573.

Semagn, K., Y. Beyene, M. L. Warburton, A. Tarekegne, S. Mugo, B. Meise, P. Sehabiague, and B. M. Prasanna. 2013. Meta-analyses of QTL for grain yield and anthesis silking interval in 18 maize populations evaluated under water-stressed and well-watered environments. BMC Genomics. 14:313.

Taylor, R. L., Jr., 2004. Major histocompatibility (B) complex control of responses against Rous sarcomas. Poult. Sci. 83:638–649.

Touko, B. H., C. T. Keambou, J. M. Han, C. Bembidé, R. A. Skilton, M. Ogugo, Y. Manjeli, S. Osama, C. Y. Cho, and A. Djikeng. 2015. Molecular typing of the major histocompatibility complex B microsatellite haplotypes in Cameroon chicken. Anim. Genet. Resour. 56:47–54.

Uni, Z., D. Sklan, N. Haklay, N. Yonash, and D. Heller, 1995. Response of three class-IV major histocompatibility complex haplotypes to Eimeria acervulina in meat-type chickens. Br. Poult. Sci. 36:555–561.

Worley, K., M. Gillingham, P. Jensen, L. J. Kennedy, T. Pizzari, J. Kaufman, and D. S. Richardson. 2008. Single locus typing of MHC class I and class II B loci in a population of red jungle fowl. Immunogenetics 60:233–247.

Yonash, N., L. D. Bacon, R. L. Witter, and H. H. Cheng. 1999. High resolution mapping and identification of new quantitative trait loci (QTL) affecting susceptibility to Marek's disease. Anim. Genet. 30:126–135.

Downloaded from https://academic.oup.com/ps/advance-article-abstract/doi/10.3382/ps/pez431/5543517 by EKU Libraries user on 04 August 2019