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Reproductive Characteristics Differed in a Series of B-Congenic Lines of White Leghorns Evidently Indicating Significant Influence of Major Histocompatibility Complex in Chickens

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Abstract: Reproductive characteristics were compared between a series of seven B-congenic lines of White Leghorns retrospectively using eight year's reproduction records. The records encompass a total of 33,010 fertile eggs set to hatch under controlled conditions, which were collected accumulatively from 2,129 young hens on the USDA-ARS, Avian Disease and Oncology Laboratory specific pathogen free farm during the years (2008-2015). Over the eight year period, average fertility, embryo mortality and hatchability of fertile eggs ranged from 71.6±0.77 (line 15.N-21) to 88.2±0.56 (line 15.P-19), 18.6±0.83 (line 15.6-2) to 28.5±0.84 (line 15.N-21) and 41.2±0.92 (line 15.N-21) to 57.6±1.09 (line 15.6-2) between the B-congenic lines and from 73.0±0.93 (2014) to 87.2±0.58 (2011), 19.5±0.84 (2012) to 35.2±0.97 (2009) and 38.3±1.02 (2009) to 55.4±1.12 (2014) between the years, respectively. Statistical analyses showed both B-congenic line (genetics) and the year of line reproduction (environmental) as well as the line by year (genetics by environment) interaction significantly influenced the reproductive characteristics ($p<0.0001$). Considerable variation observed between the B-congenic lines each year suggests the reproductive performance followed a polygenic model of inheritance, which are attributable to genes of the major histocompatibility complex (MHC) loci. Findings from this study document the reproductive characteristics of the 7 B-congenic lines of White Leghorns and add to the mounting evidence that MHC affects reproductive characteristics in a diverse species including human, wild chimpanzees, fish, bovine, swine, mice, rats and chickens.

Key words: White leghorns, B-congenic lines, MHC, genetics, fertility, embryo mortality, hatchabiity

INTRODUCTION

Reproductive performance is essential for commercial and experimental lines of chickens not only for line reproduction itself but also for production (commercial lines) and timely producing adequate number of chicks (experimental lines) to meet research needs. Fertility, embryo mortality and hatchability of fertile eggs are among the major estimates of parameters for reproductive performance of egg-layer type of chickens. Unfortunately, the estimates of heritability for these reproductive traits in chickens are reportedly low, ranging from 0.05 to 0.13, which indicate environmental factors, such as management measures, nutrient supplies, farm housing conditions and yearly climate factors, impose significant influence on these important traits of chickens (Stromberg, 1975; Cahaner and Hillel, 1980; Bacon *et al*., 1985; Brah *et al*., 1991; Abplanalp *et al*., 1992; Abplanalp, 1992; Bacon *et al*., 2001; Sapp *et al*., 2004; King'ori, 2011; Wright *et al*., 2012).

Recently after careful examination of a subset of 8-year reproduction records for 21 highly inbred lines of White Leghorns, which were developed and have been maintained on the specific pathogen free (SPF) farm of the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS), Avian Disease and Oncology Laboratory (ADOL) at East Lansing, Michigan, we reported that polygenic effects play a substantial role in controlling of the reproductive characteristics in the 21 inbred lines of chickens. Furthermore, the polygenic effects detected in the 21 inbred lines were primarily, if not completely, contributable to genes outside of the major histocompatibility complex (MHC) of the chicken genome since all of the 21 inbred lines of chickens share a single MHC type, the B*2 haplotype (Bacon *et al*., 2000; Kulkarni and Zhang, 2015).

The chicken MHC was first recognized from work on chicken blood group system. Therefore, the general symbol of the MHC gene loci was designated as B due to the link at the discovery processes between MHC and what it was thought as the second chicken blood group system (Briles *et al*., 1950; Gilmour, 1959; Lamont, 1998). The chicken B system was the first comprehensively characterized MHC system in vertebrates (Lamont, 1998; Miller *et al*., 2004; Kaufman,

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2015). Chicken MHC system is depicted as a simple and compact system, yet consisting of 11 common (B*2, B*4, B*5, B*6, B*7, B*12, B*13, B*14, B*15, B*19 and B*21) and 16 less common (B*1, B*3, B*8, B*9, B*10, B*11, B*17, B*18, B*22, B*23, B*24, B*25, B*26, B*27, B*28 and B*29) B-haplotypes based on the blood typing data of 121 individual chickens using a total of 80 MHC B-serological reagents collectively offered by a dozen of research laboratories (Kaufman, 2000; Miller *et al*., 2004) during that process.

The B-system are reportedly associated with immune responses (Antczak, 1982; Nordskog *et al*., 1987; Lamont, 1991; Plachy *et al*., 1992; Kaufman *et al*., 1999; Owen *et al*., 2009; Nikbakht and Esmailnejad, 2015), resistance to diseases, including Marek's disease, avian leukosis, necrotic enteritis, bacterial infections and mite infestations (Bacon *et al*., 1985; Nordskog *et al*., 1987; Lamont, 1991; Kaufman and Salomonsen, 1992; Plachy *et al*., 1992; Yoo and Sheldon, 1992; Siegel *et al*., 1993; Bacon and Witter, 1994; Kaufman and Wallny, 1996; Wakenell *et al*., 1996; Lakshmanan *et al*., 1997; Lamont, 1998; Bacon *et al*., 2000; Kaufman, 2000; Hunt *et al*., 2001; Owen *et al*., 2009), vaccine efficacy (Bacon and Witter, 1994; Bacon and Witter, 1995; Pharr *et al*., 1998; Kaufman, 2000; Bacon *et al*., 2004), autoimmune disease (Lamont, 1991), onset of sexual maturity and sex ratio (Lunden *et al*., 1993; Wright *et al*., 2012; Nikbakht and Esmailnejad, 2015), feed efficiency (Lamont *et al*., 1987), egg production (Lamont *et al*., 1987; Abplanalp *et al*., 1992; Yoo and Sheldon, 1992; Sato *et al*., 1992; Lunden *et al*., 1993; Siegel *et al*., 1993; Cahaner *et al*., 1996; Lakshmanan *et al*., 1997; Owen *et al*., 2009; Wright *et al*., 2012) in chickens. MHC was reportedly associated with reproductive characteristics in human, wild chimpanzees, fish, bovine, swine, mice, rats and chickens (Gill *et al*., 1984; Lamont *et al*., 1987; Mallard *et al*., 1987; Nordskog *et al*., 1987; Singh and Verma, 1987; Renard and Vaiman, 1989; Stear *et al*., 1989; Gautschi and Gaillard, 1990; Abplanalp *et al*., 1992; Sato *et al*., 1992; Liljander *et al*., 2006; Porsova-Dutoit, 2006; Jager *et al*., 2007; Gillingham *et al*., 2009; Lovlie *et al*., 2013; Lynge *et al*., 2014; Wroblewski *et al*., 2015).

Lamont *et al*. (1987) examined the relationships between B-haplotypes and growth characteristics as well as reproduction traits in lines of White Leghorn chickens that were subjected to long term selection for growth and reproduction traits. After 6 generation of selection, the data from that study suggested selection for growth, feed efficiency and reproduction significantly increased the B*2 but decreased B*13 haplotype in the lines of chickens. Abplanalp *et al*. (1992) assessed the production and reproduction performance of twelve congenic lines of White Leghorns and found that the highest average fertility was observed in the chicken line homozygous for B*19/*19 (92.2%) haplotype and the lowest fertility in a chicken line homozygous for B*3/*3 (79.7%) haplotype; the highest hatchability of fertile eggs was observed in a chicken line homozygous for B*C/*C (79.8%), one of B-haplotype described by Miller *et al*. (1988) and the lowest average hatchability, in a chicken line homozygous for B*17/*17 (52.9%) haplotype. Egg production, egg weight, body weight and hen housed mortality also varied among the B-congenic lines. This study was aimed to document the reproductive characteristics of the B-congenic lines, formally known as 15.B-congenic lines and to evaluate genetics and environmental factor's influence on the reproductive characteristics of the lines, which were also developed and have been maintained at the USDA-ARS, Avian Disease and Oncology Laboratory at East Lansing, Michigan, for evaluation of MHC influences on disease resistance and immune responses to tumor viruses.

MATERIALS AND METHODS

Chicken lines: Reproductive records sampled to evaluate the reproductive performance were from the progenitor line $15I_5$ and the series of 15.B-congenic lines. The developmental details of the progenitor line $15I_5$ and its B-congenic line series (15.6-2, 15.C-12, 15.P-13, 15.P-19 and 15.N-21) were reviewed by Bacon *et al*. (2000). Briefly, each of the 15.B congenic lines was initiated by crossing a donor line of distinct B-haplotype to the progenitor background line $15I_5$ followed by backcrossing the heterozygous males to the $15I_5$ hens for 10 or 11 consecutive generations. The donor lines for the B-congenic lines 15.6-2, 15.C-12 and 15.N-21 were line 6_3, RH C and N, respectively; the donor line for both 15.P-13 and 15.P-19 was the ADOL line P. After the lines were established, the line $15I_5$ chickens are histocompatible, 99% inbred, susceptible to avian leukosis viruses (subgroups A, B, D, E and J) and Marek's disease viruses, carrying avian subgroup E endogenous virus genes, which include ev 1, 6 and 10 and chicken MHC B*15 haplotype genes. Each of the 15.B-congenic lines is 99.9% homologous to the inbred progenitor background line $15I_5$ and each is homozygous for a unique B-haplotype linked to the nucleolar organizer region on a micro-chromosome (Bacon *et al*., 2000). The B-haplotypes carried by the progenitor line ($15I_5$) and the B-congenic lines (15.6-2, 15.C-12, 15.P-13, 15.P-19 and 15.N-21) are B*15, B*2, B*12, B*13, B*19 and B*21, respectively.

Line reproduction schemes: The progenitor line $15I_5$ and the B-congenic lines are reproduced once a year under similar mating and hatching schemes. Line $15I_5$ is reproduced each year by selecting 6 pedigreed full and/or half sib males that each is mated to 7 random females of the line via artificial insemination. For each of the B-congenic lines, fresh semen collected from 6 selected males are pooled to artificially inseminate 42

females. Semen samples are collected and artificial inseminations are carried out twice each week during a reproduction season, which starts early each year when the breeders reach about 35 weeks of age. Hatch eggs are collected daily and are individually marked with cage numbers. Freshly collected eggs are disinfected with Lysol solution prior to being placed into storage coolers, which are set to 15.6°C and 55% relative humidity. Collected eggs are set to hatch weekly. The eggs set to hatch are candled at 10-12 days of incubation. Both infertile eggs and dead embryos are recorded and removed. The remaining embryos are remained for incubation until hatched on the 21st day. Chicks are individually tagged for identification at hatch using wing-bands and are recorded.

Reproduction data: Records collected from year 2008 to year 2015 were sampled to evaluate the reproduction characteristics of the B-congenic lines of chickens. Fertility was calculated as the ratio of the number of fertile eggs divided by the total number of eggs set for incubation multiplied by 100; embryo mortality (EM) was defined as the number of dead embryos determined during candling around 10-12 days of incubation divided by the total number of embryos multiplied by 100; hatchability refers to the percentage of fertile eggs that hatched, which was calculated as the ratio of the number of hatched chicks divided by the total number of embryos (dead or alive determined at candling) multiplied by 100. The numbers of hens and eggs set to hatch that were included in this study for each chicken line and year of reproduction are given in Table 1.

Statistical analysis: The records of the dataset were filtered first to remove any record of hens that had fewer than 3 eggs in a set for incubation. The percentage data for fertility, embryo mortality and hatchability of fertile eggs were subjected to square root transformation to normalize the residuals prior to fit a full general linear model, Y = Line+Year+Line*Year, by which the chicken line, year of reproduction and the chicken line by year of reproduction interaction effects on the Y variables were statistically tested, where the Y refers to fertility, embryo mortality and hatchability of fertile eggs. The statistical significance of the chicken line, year of reproduction and line by year interaction effects was examined by F test under the linear model. Pairwise comparisons among the lines of chickens within each of, as well as across, the years for each of the three variables, fertility, embryo mortality and hatchability of fertile eggs, were also tested under the linear model for statistical significance using the Duncan's multiple-range test. For direct and easy interpretation, however, the averages of fertility, embryo mortality and hatchability of fertile eggs for each of the lines in each of the years were tabulated and plotted with the data prior to the square root

Table 1: Number of hens and eggs set to hatch included in the samples from ADOL farm records for a retrospective evaluation on reproductive performance of a series of B-congenic lines of White Leghorns

	Year																	
	2008		2009		2010		2011		2012		2013		2014		2015		Total	
Line	Hens	Eggs	Hens	Eggs	Hens	Eggs	Hens	Eggs	Hens	Eggs	Hens	Eggs	Hens	Eggs	Hens	Eggs	Hens	Eggs
$15I_5$	42	715	33	241	39	833	42	595	38	546	40	772	41	544	41	757	316	5,003
15.6-2	33	387	34	352	36	720	36	582	39	459	37	465	37	430	36	431	288	3,826
15.15I-5	41	388	39	773	46	731	45	884	43	470	51	641	36	569	56	774	357	5,230
15.C-12	35	472	33	608	35	453	33	538	31	360	38	469	39	467	39	551	283	3,918
15.P-13	36	518	36	677	34	717	36	824	39	497	41	689	41	762	36	493	299	5,177
15.P-19	35	436	33	702	34	461	36	654	33	484	39	542	39	516	36	496	285	4,291
15.N-21	36	704	36	855	36	588	35	627	37	612	41	665	40	839	40	675	301	5,565
Total	258	3,620	244	4,208	260	4,503	263	4,704	260	3,428	287	4,243	273	4,127	284	4,177	2,129	33,010

transformation (Table 2-4; Fig. 1-3). All statistical analyses were performed using the JMP® 12 SAS package (SAS Institute Inc., 2015).

RESULTS AND DISCUSSION

The primary objective of this study was to retrospectively characterize and document reproductive performance of the series of experimental 15.B-congenic lines of White Leghorns for the first time since the establishment in 1988 and to statistically evaluate genetics and environmental influence over the reproductive characteristics.

Fertility: Table 2 gives the average fertility of set eggs to hatch for each line each year during the eight years. The average fertility for all the lines over the entire period of this study was 80.8±0.28% with considerable variation between the lines, ranging from 71.6±0.77 (Line 15.N-21, B*21/*21) to 88.2±0.56 (line 15.P-19, B*19/*19) and between the years, ranging from 73.0±0.93 (year 2014) to 87.2±0.58 (year 2011). Of the progenitor line $15I_5$ and its 6 B-congenic lines, the line 15.P-19 was observed with the highest fertility throughout the eight years, with average fertility ranging from 82.6±2.79 (year 2008) to 91.4±2.74 (year 2015), which were statistically comparable to the progenitor line $15I_5$ ($p>0.05$), except in year 2008 and very significantly higher than the line 15.N-21 within each or across of the years ($p<0.001$). The rest of the lines fell somewhere in between the lines 15.P-19 and 15.N-21 (Table 2, Fig. 1). Since each of the 15.B congenic lines is 99.9% identical to its progenitor background line $15I_5$, but each is homozygous for unique genes of one common B-haplotype (Bacon *et al*., 2000), for instance, the lines 15.P-19 and 15.N-21 are B*19 and B*21, respectively, thus, the considerable variation between the lines suggests significant polygenic effects underlying fertility, which were primarily, if not entirely, attributable to MHC genes on the 7 common B-haplotype loci. The finding that B*19 chickens (15.P-19) had the highest fertility of fertile eggs in this study is in good agreement with the report of a study also conducted in a different set of B-congenic lines of White Leghorn over two decades ago. But the lowest fertility of the chicken line in that study was not B*21 but B*3 (Abplanalp *et al*., 1992). This inference agrees in principal with an earlier report on genetic influence over reproductive characteristics of 21 highly inbred lines of White Leghorns sharing a common (B*2) haplotype (Kulkarni and Zhang, 2015).

Embryo mortality: Table 3 lists the average embryo mortality rates for each of the lines in each year during the eight years. The average embryo mortality for all lines over the entire period of this study was 24.5±0.31 percent with considerable variation between the lines, ranging from 18.6±0.83 (line 15.6-2) to 28.5±0.84 (line

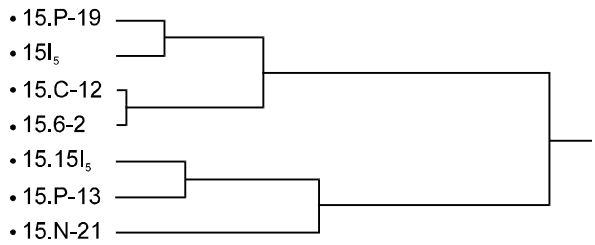

Fig. 1: A hierarchical clustering tree depicts the relative rank of the progenitor line $15I_5$ and the B-congenic lines in fertility realized during a period of eight years (2008-2015). The line 15. P-19 had the highest observed average fertility among all the lines followed by the progenitor line $15I_5$, which significantly differed from most of the lines ($p<0.05$). The lines 15.C-12 and 15.6-2 were observed with average fertilities that fell around the middle of the range and did not differ from each other ($p>0.05$) but from others ($p<0.05$). Line $15.15I_5$ had a relatively lower fertility and differed from every other line ($p<0.05$). Line 15.P-13 was ranked next to the lowest line 15.N-21 in average fertility, but differed from each other and both differed from each of the other lines ($p<0.05$)

15.N-21) and between the years, ranging from 19.5±0.84 (year 2012) to 35.2±0.97 (year 2009). Line 15.6-2, the B*2 haplotype congenic line, was observed with the lowest or comparable to the lowest embryo mortality rates among the lines throughout the eight years except in year 2011; in that year, the line 15.N-21 was ranked the lowest embryo mortality. In the rest of years, line 15.N-21, the B*21 haplotype line, was ranked the highest (2008-2010 and 2012-2014) or comparable to the highest embryo mortality (lines 15.P-19 and 15.P-13 in year 2015). The embryo mortality rates for rest of the lines during most of the years fell somewhere in between of the lines 15.6-2 and 15.N-21. The relative ranking of embryo mortality for the lines during the entire period of this study was depicted in Fig. 2. As it shows, the progenitor line $15I_5$ (B*15 haplotype) was ranked comparably next to the lowest embryo mortality line 15.6-2. The observed variation between the lines suggests a similar polygenic model of inheritance, as did for fertility and the fluctuation of embryo mortality between the years indicated significant environmental influence, which are coincided with theoretical expectations of the reproductive characteristics.

Hatchability of fertile eggs: Table 4 tabulates the average hatchability of fertile eggs for each line in each year during the eight years. The average hatchability of fertile eggs for all the lines over the entire period of this study was 47.9±0.3% with considerable variation between the lines, ranging from 41.2±0.92 (line 15.N-21)

Table 2: Average fertility (mean±standard error)[1] of the B-congenic lines of White Leghorns during the eight years

	Year							
Line	2008	2009	2010	2011	2012	2013	2014	2015
$15I_5$	73.5±2.48^{bc}	91.2±2.85^{a}	89.0±2.25^{a}	88.6±2.37^{ab}	87.3±2.62^{ab}	87.9±2.00^{a}	88.7±2.43^{a}	88.8±2.21
15.6-2	84.9±3.29^{a}	83.1±3.11^{b}	85.5±2.38^{a}	89.5±2.71^{ab}	83.0±2.61^{bc}	83.8±3.23^{ab}	69.4±3.46^{bc}	84.1±3.09
$15.15I_5$	78.2±3.33^{ab}	86.5±1.99^{ab}	79.0±2.24^{b}	86.4±2.12^{b}	84.9±2.87^{abc}	67.7±2.58^{d}	72.6±2.88^{bc}	80.6±2.52
15.C-12	83.2±2.85^{a}	81.6±2.29^{b}	88.5±2.62^{a}	88.7±2.58^{ab}	86.2±3.39^{abc}	80.4±2.90^{bc}	79.8±3.24^{b}	81.5±2.54
15.P-13	69.2±3.47^{c}	81.0±2.60^{b}	73.8±2.52^{bc}	86.1±2.05^{b}	80.2±2.62^{cd}	77.9±2.52^{c}	66.8±2.87^{c}	83.0±3.01
15.P-19	82.6±2.79^{a}	89.8±2.30^{a}	90.5±2.68^{a}	91.2±2.33^{a}	89.8±2.54^{a}	84.3±2.59^{ab}	84.9±2.71^{a}	91.4±2.74
15.N-21	69.6±2.59^{c}	73.7±2.31^{c}	71.8±2.73^{c}	81.1±2.38^{c}	76.4±2.50^{d}	80.0±2.42^{bc}	61.0±2.63^{d}	61.1±3.04

[1]Means not sharing a common superscript letter or letters within each column are significantly different from each other ($p<0.05$) based on Duncan's multiple-range test. This note remains true for the Tables 3 and 4

Table 3: Average embryo Mortality (mean±standard error) of the B-congenic lines of White Leghorns during the eight years

	Year							
Line	2008	2009	2010	2011	2012	2013	2014	2015
$15I_5$	20.0±2.02^{b}	22.2±2.85^{b}	27.0±1.97^{ab}	22.9±1.97^{bc}	16.7±1.77^{bc}	18.4±1.45^{bc}	15.8±1.89^{b}	13.2±1.63^{d}
15.6-2	17.3±2.89^{b}	18.3±2.59^{b}	19.8±1.74^{b}	26.9±2.37^{ab}	12.5±1.93^{c}	15.8±2.15^{c}	18.7±2.75^{b}	18.1±2.31^{cd}
$15.15I_5$	15.3±2.60^{b}	37.1±2.06^{a}	30.9±2.10^{a}	36.3±2.37^{a}	19.6±2.47^{bc}	16.5±2.04^{c}	15.8±2.22^{b}	23.7±1.88^{bc}
15.C-12	19.7±2.02^{b}	37.6±2.45^{a}	22.1±2.36^{ab}	21.9±2.12^{bc}	18.4±2.34^{bc}	25.1±2.18^{ab}	22.3±2.59^{ab}	22.6±2.31^{bc}
15.P-13	20.5±2.59^{b}	39.7±2.50^{a}	31.0±2.29^{a}	22.9±1.82^{bc}	20.5±2.19^{ab}	25.7±2.05^{ab}	31.4±2.58^{a}	27.6±2.79^{ab}
15.P-19	14.6±2.02^{b}	38.5±2.39^{a}	24.2±2.40^{ab}	25.2±2.03^{b}	18.4±2.00^{bc}	29.7±2.55^{a}	20.8±2.10^{ab}	32.8±2.55^{a}
15.N-21	31.8±2.28^{a}	39.1±2.34^{a}	29.6±2.68^{ab}	19.3±1.89^{c}	26.8±2.25^{a}	27.4±2.24^{ab}	25.0±2.32^{ab}	27.6±2.63^{ab}

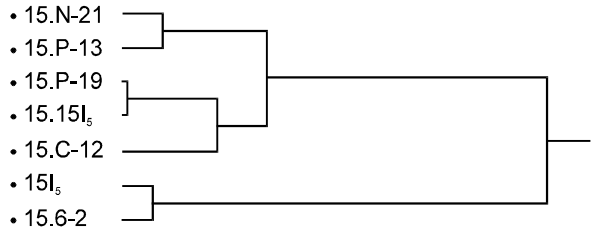

Fig. 2: A clustering tree depicts the relative rank of embryo mortality among the B-congenic lines. The progenitor line $15I_5$ and the B-congenic line 15.6-2 were ranked in the lowest group in embryo mortality and were not different from each other ($p>0.05$), but differed from the rest of the lines ($p<0.05$). Line 15.C-12 was ranked next to the lowest group and differed from each of the two lines ($15I_5$ and 15.6-2). Both lines 15.N-21 and 15.P-13 had the highest embryo mortality and not statistically differed from each other as well as lines 15.P-19, $15.15I_5$ ($p>0.05$), but the rest of lines ($p<0.05$)

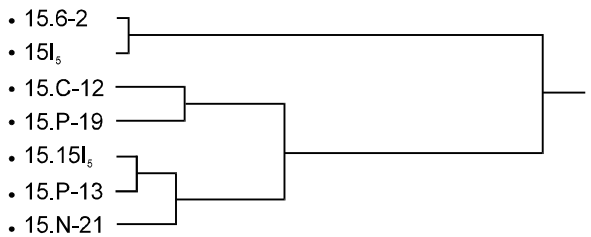

Fig. 3: A clustering tree depicts the relative rank of average hatchability of fertile eggs among the 7 B-congenic lines. On the contrary to embryo mortality (Fig. 2), both the progenitor line $15I_5$ and the B-congenic line 15.6-2 were ranked in the highest hatchability group, not differed from each other ($p>0.05$) but from rest of the lines ($p<0.05$); the line 15.N-21 was ranked as the lowest in hatchability among all the lines ($p<0.05$). Both lines $15.15I_5$ and 15.P-13 were ranked next to the lowest line, not different from each other but differed from each of the rest of lines. Lines 15.C-12 and 15.P-19 fell between the middle of the range in hatchability, differed from each other and the rest of the lines ($p<0.05$)

to 57.6±1.09 (line 15.6-2) and between the years, ranging from 38.3±1.02 (year 2009) to 55.4±1.08 (year 2014). In contrast to embryo mortality, it is interesting to note that the line 15.6-2 was ranked the highest in hatchability of fertile eggs throughout the years except the year of 2011, in which, the line $15I_5$ was ranked the highest in hatchability. Line 15.N-21 was ranked the lowest, except in the year 2011. In that year, lines 15.6-2 and 15.N-21 were comparable to each other in hatchability ($p>0.05$). The relative hatchability of fertile eggs between the lines during the period of this entire study was depicted in Fig. 3. The significant difference in hatchability of fertile eggs observed between the B*2 and B*21 haplotype lines (15.6-2 and 15.N-21, respectively) is, however, inconsistent with Abplanalp *et al*. (1992), in which hatchability of fertile eggs was found significantly varied between some B-congenic lines but insignificantly different between the B*2 and B*21 haplotype lines in their study. The results of this study suggest fertility of fertile eggs also followed a polygenic model of inherence, just as did the fertility and embryo mortality of the B-congenic lines and was also under significant influence of environmental factors, which resulted in considerable variability between the years in almost all of the lines.

Table 4: Average hatchability of fertile eggs (mean±standard error) of the B-congenic lines of White Leghorns during the eight years

	Year							
Line	2008	2009	2010	2011	2012	2013	2014	2015
$15I_5$	60.8±2.48^{a}	60.7±2.85^{a}	44.8±2.25^{b}	48.3±2.37^{a}	52.3±2.62^{b}	58.4±2.00^{a}	67.3±2.43^{a}	64.6±2.21^{a}
15.6-2	63.8±3.29^{a}	63.7±3.11^{a}	53.7±2.38^{a}	37.9±2.71^{bc}	66.0±2.61^{a}	60.2±3.23^{a}	62.7±3.46^{ab}	56.7±3.09^{b}
$15.15I_5$	59.0±3.33^{a}	28.9±1.99^{b}	37.6±2.24^{c}	34.4±2.12^{c}	55.0±2.87^{ab}	61.0±2.58^{a}	51.3±2.88^{bc}	40.4±2.52^{d}
15.C-12	58.8±2.85^{a}	34.9±2.29^{b}	45.5±2.62^{b}	43.3±2.58^{ab}	57.0±3.39^{ab}	55.2±2.90^{a}	53.7±3.24^{bc}	50.6±2.54^{bc}
15.P-13	50.4±3.47^{b}	36.7±2.60^{b}	37.9±2.52^{c}	34.8±2.05^{c}	52.0±2.62^{b}	43.7±2.52^{b}	46.3±2.87^{c}	49.7±3.01^{bc}
15.P-19	61.6±2.79^{a}	33.9±2.30^{b}	47.1±2.68^{b}	38.5±2.33^{bc}	57.1±2.54^{ab}	41.5±2.59^{bc}	59.4±2.71^{ab}	39.4±2.74^{d}
15.N-21	37.8±2.59^{c}	33.3±2.31^{b}	42.3±2.73^{bc}	41.1±2.38^{bc}	41.3±2.50^{c}	36.5±2.42^{c}	51.7±2.63^{bc}	45.6±3.04^{cd}

The 15.B-congenic lines constitute part of the critical genetic resources of well-characterized chicken lines developed and having been maintained at the USDA-ARS, Avian Disease and Oncology Laboratory at East Lansing, Michigan, U.S.A. The 15.B-congenic lines were developed primarily to assess MHC influence on genetic resistance to Marek's disease and immune response (Bacon *et al*., 2000). By use of these lines of chickens, it has been demonstrated that MHC B-haplotypes do significantly contribute to disease resistance and vaccine protective efficacy (Bacon *et al*., 1981; Bacon *et al*., 1983; Bacon and Witter, 1995; Bacon *et al*., 2001; Bacon *et al*., 2004; Mays *et al*., 2005). In addition, the B-congenic lines of chickens were also used in assessments of MHC influence on susceptibility and immune response to avian influenza viruses (Hunt *et al*., 2010; Kapczynski *et al*., 2011). The findings of this study on the reproductive characteristics of the 15.B-congenic lines of White Leghorns in general are in good agreement with reports that MHC B-haplotype genes do affect reproductive performance in species including human, livestock and poultry (Gill *et al*., 1984; Singh and Verma, 1987; Mallard *et al*., 1987; Renard and Vaiman, 1989; Stear *et al*., 1989; Gautschi and Gaillard, 1990; Brah *et al*., 1991; Abplanalp *et al*., 1992; Liljander *et al*., 2006; Jager *et al*., 2007; Gillingham *et al*., 2009; Wright *et al*., 2012; Lovlie *et al*., 2013; Lynge *et al*., 2014).

Abbreviations key: ADOL (Avian Disease and Oncology Laboratory), EM (Embryo mortality), AI (Artificial insemination), MHC (Major histocompatibility complex), SPF (specific pathogen free) USDA-ARS (U.S. Department of Agriculture, Agricultural Research Service).

Conclusion: In summary, this report documents the reproductive characteristics of a highly inbred progenitor line $15I_5$ (B*15) of White Leghorns and its 6 B-congenic lines with the estimates of average fertility, embryo mortality and hatchability of fertile eggs. Results from the statistical analyses of the dataset showed the genetic line, the year in which the reproduction of the B-congenic lines took place and an interaction between the genetic line and the year significantly affected each of the reproductive characteristics examined. The highest average of fertility during the period of this entire study was observed in the B*19 haplotype (15.P-19) line while the B*21 haplotype (15.N-21) line was the lowest one. In addition, the B*21 haplotype line was observed with the highest embryo mortality and lowest hatchability, while the B*2 haplotype (15.6-2) line had the lowest embryo mortality and highest hatchability of fertile eggs.

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