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Evaluation of Reproductive Characteristics of 21 Highly Inbred Lines of White Leghorns Divergently Selected for or Segregating in Tumor Resistance

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Abstract

Reproduction performance of 21 inbred experimental lines of White Leghorns was evaluated based on samples of reproduction records over a period of eight consecutive years. Two lines (6_3 and 7_2) have been extensively used in studies, especially in research seeking for genetic and epigenetic factors underlying resistance to avian tumor virus-induced diseases in chickens. The other 19 lines are recombinant congenic strains (RCS), which were generated by crossing lines 6_3 and 7_2 followed by two consecutive backcrosses to the line 6_3 and then full-sib mating. In theory, each RCS processes 7/8 of progenitor background line 6_3 genome and a random sample (1/8) of the progenitor donor line 7_2 genome. All 21 inbred lines share a common major histocompatibility complex haplotype, *B2. The estimated average fertility of the 21 inbred lines ranged from 72.9% (RCS-J) up to 96.8% (RCS-P). Both progenitor lines 6_3 and 7_2 were observed with lower average fertility (82.4% and 81.6%, respectively) in comparison with the RCS except the RCS-J, suggesting a substantial polygenic component underlying the fertility phenotype. The average embryo mortality rate ranged from 14.5% (RCS-P) up to 47.0% (RCS-M). The background line 6_3 fell at about the middle of the range (28.3%) significantly higher than the donor line 7_2 (15.7%), which was among the group with the lowest embryo mortality. By definition, hatchability of fertile eggs is reversely correlated with embryo mortality. The average hatchability ranged from 26.5% (RCS-M) up to 66.8% (line 7_2) while the background line 6_3 remained (46.6%) at about the middle of the range. The variability of the average embryo mortality and hatchability observed among the 21 inbred lines indicated the two correlated traits also follow polygenic models of inheritance. Findings from this study paves the way for further investigation on genetic and environmental influence over reproductive performance of inbred lines of chickens, and particularly in understanding and improving the reproduction fitness of invaluable genetic resources like these inbred lines.**

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Keywords

White Leghorns, Inbred Lines, Recobinant Congenic Strains, Fertility, Embryo Mortality, Hatchabiity, Polygentic Inheritance

1. Introduction

Reproduction characteristics are of profound importance for livestock and poultry both in evolutionary and economic perspectives. Fertility and hatchability are major parameters of reproductive performance for egg-layer type of chickens. The heritability estimates for fertility, embryo mortality, and hatchability of fertile eggs in chickens reportedly range from 0.05 to 0.13, which indicate these important traits bear heavy influence from management measures, nutrient supplies, and surrounding environmental conditions [1]-[5].

Since the foundations for genetic improvement were laid by Robert Bakewell in the 18th century [6], a variety of ways to employ inbreeding in combination with intensive selection to achieve breeding goals has been attempted. The adverse effects of inbreeding, commonly referred to as inbreeding depression, are also richly documented. In poultry, inbreeding depression not only adversely affect production performance, but also reproductive characteristics, including fertility, embryo mortality, and hatchability [7]-[13].

The adverse consequence resulted from inbreeding, particularly severe in closed small populations of domesticated poultry, leads to partial or complete loss of poultry lines or flocks from time to time [12]. For instance, five out of a series of 24 recombinant congenic strains (RCS) of White Leghorns, initiated and maintained at the USDA, Agriculture Research Service (USDA-ARS) laboratory at East Lansing, Michigan, completely failed to reproduce by the sixth generation of full-sib mating [14]. Inbreeding escalates homozygosity and speeds up fixation, by chance, of deleterious recessive alleles simultaneously at multiple loci. Due to natural or artificial selection, such recessive alleles generally remain with low or very low frequencies at heterozygous state in outbred populations, and their harmful effects are hardly detectable phenotypically. However, when inbreeding takes place, the chance for homozygosity of such deleterious alleles is drastically increased. Especially when inbreeding occurs consecutively over generations, homozygosity of such alleles at multiple loci generally show up at high frequencies, which would have significant negative impact on production, reproduction, and fitness characteristics of the populations [6] [8] [11] [12] [15] [16].

Despite of the hurdles and barriers along the way in developing inbred animals, inbred, recombinant inbred, and/or recombinant congenic inbred strains have been generated in many organisms including mice, cattle, and chicken [10] [14] [17]. Inbred lines, especially characterized inbred lines of animals are invaluable resources for genetic studies [17]-[21]. In particular, many unique genetic lines of chickens were developed and thirty five of which have been maintained at the USDA-ARS, Avian Disease and Oncology Laboratory (ADOL) at East Lansing, Michigan. Two-thirds of the genetic lines are highly inbred lines or RCS. Chickens from all of the lines have been serving as critical resources for successful completion of important projects in the past and remains being critically vital for new projects that are conducted in the genomic and post-genomic era at ADOL. The ADOL inbred lines as well as outbred lines of chickens also have been one of the important sources for research and diagnostic tests at other research centers and laboratories in the United States and around the world. The National Animal Germplasm Program (NAGP; http://www.ars.usda.gov/Research/docs.htm?docid=22314) approved and collected all chicken lines developed at ADOL, including the thirty five lines that are currently maintained at ADOL. This study was aimed to provide estimates on fertility, embryo mortality, and hatchability of fertile eggs for 21 out of the 35 lines, which include two highly inbred lines of chickens and a series of 19 RCS using sampled reproduction records during a period of eight years.

2. Materials and Methods

2.1. Lines of Chickens

A total of 21 inbred experimental lines of White Leghorn was included in this study to examine reproduction characteristics. Two of which, the line 6_3 and line 7_2, are highly inbred and diversely selected for tumor resistance. Line 6_3 is resistant to Marek's disease (MD) and line 7_2 is highly susceptible to MD. Both lines 6_3 and 7_2

share the same major histocompatibility complex (MHC) haplotype, *B**2. The other 19 lines are a series of RCS derived from the lines 6_3 and 7_2 by crossing the two lines followed with two consecutive backcrosses to the background line 6_3. The details in development of all the 21 lines of chickens were reviewed by Bacon *et al.* [14].

2.2. Reproduction Data

Records collected from Year 2005 to Year 2012 were sampled to evaluate the reproduction characteristics of the inbred genetic lines of chickens. The line reproduction each year during the 8 years began with artificial insemination (AI) during January to March. AI and incubation handlings were all accomplished by a team of same four people during the period of time. A total of 84 hens and 12 males from each of lines 6_3 and 7_2 were used for line reproduction. The 12 males were selected from two families, six from each, within each of the lines 6_3 and 7_2. Each male was mated to 7 random females. For the RCS, a total of 21 hens and 2 full-sib males for each line were used for reproduction. One male was mated to 10 females, and the other, to 11 females. The reproduction performance was measured by collecting the eggs for incubation from 1 to 2 weeks at around 35 weeks of age for each hatch for a total of three hatches per laying hen for each line. Fertility was calculated as the ratio of number of fertile eggs divided by the total number of eggs set for incubation multiplied by 100; the embryo mortality (EM) was defined as the number of dead embryos determined during candling around 8 - 9 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 records included in this study for each line and year are listed in **Table 1**.

2.3. Statistical Analyses

The records of the dataset were filtered first to remove any record of hens that had fewer than 3 eggs at the time 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 a reduced general linear model, $Y_{ij} = Year_i + Line_j + e$, was fitted to examine the year and chicken line effects on the Y variables, where Y refers to the fertility, embryo mortality, hatchability of fertile eggs, and the e refers to the residual error term. The statistical significance of the year and chicken line effects was examined by F test under the linear model. The 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, 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 transformation (**Tables 2-4**). All statistical analyses were performed using the JMP® 11 SAS package [22].

3. Results

3.1. Fertility

Fertility measures the capacity of poultry to initiate its reproduction process. The variability of fertility from year to year during the 8 years for all the inbred lines was statistically significant ($P < 0.0001$). The average fertility of all the lines ranged from 81.7% ± 0.6% in one year (2012) to 91.6% ± 0.5% in another (2010). Statistically significant difference was also detected for average fertility over the eight years between the inbred experimental lines ($P < 0.0001$) with an even larger range between 72.9% ± 1.3% for RCS-J and 96.8% ± 0.4% for RCS-P. Examining the average fertility by line over the eight years, the average fertilities of the progenitor lines 6_3 and 7_2 were ranked on the lower side with averages of 82.4% ± 0.6% and 81.6% ± 0.8%, respectively, and were not significantly different from each other ($P > 0.05$; **Table 2**). A hierarchical clustering tree was constructed to graphically illustrate the relative average fertility ranks among the 21 inbred lines over the eight year period (**Figure 1**).

Table 2 also lists the average fertility estimates for by line and year for each of the 21 inbred experimental lines of chickens in Year 2005 to Year 2012. The average fertilities for RCS-P and RCS-B were between 91% to 98% and were consistently ranked on the higher side among the 21 inbred lines ($P < 0.05$), and were signifi-

Table 1. Numbers of hens and set eggs sampled from ADOL farm records for the retrospective evaluation.

Line	Sampled records by year																Total	
	2005		2006		2007		2008		2009		2010		2011		2012			
	Hens	Set eggs	Hens	Set eggs	Hens	Set eggs	Hens	Set eggs	Hens	Set eggs	Hens	Set eggs	Hens	Set eggs	Hens	Set eggs	Hens	Set eggs
Line 6_3	77	1114	72	942	81	1705	78	1307	66	718	72	969	80	1330	78	985	604	9070
Line 7_2	65	680	73	746	72	1139	65	737	57	638	74	746	64	591	59	612	529	5889
RCS-A	19	307	17	205	21	257	20	276	19	257	20	277	20	242	25	330	161	2151
RCS-B	21	338	21	335	21	275	20	333	21	420	21	403	21	425	21	378	167	2907
RCS-C	20	298	21	340	19	260	21	329	20	408	20	333	20	323	20	333	161	2624
RCS-D	21	253	21	350	19	236	20	323	21	449	17	277	21	326	20	301	160	2515
RCS-F	19	370	20	311	19	358	20	377	19	427	21	323	18	273	21	385	157	2824
RCS-G	20	337	17	242	20	318	21	304	13	147	20	335	21	320	27	414	159	2417
RCS-I	16	244	21	287	20	340	17	275	20	434	21	329	19	309	19	266	153	2484
RCS-J	18	368	16	264	18	291	20	302	21	309	21	407	21	462	21	395	156	2798
RCS-K	18	304	18	319	18	296	21	360	19	308	21	403	21	402	21	347	157	2739
RCS-L	17	281	18	260	17	294	21	337	20	290	21	358	21	343	21	298	156	2,461
RCS-M	18	307	17	234	16	268	19	334	19	228	20	354	19	288	28	375	156	2388
RCS-N	17	280	16	219	18	311	19	242	20	187	21	225	19	243	17	185	147	1892
RCS-P	17	322	17	279	18	347	21	331	21	481	21	366	21	370	21	312	157	2808
RCS-R	17	257	15	175	14	209	20	318	19	335	19	288	21	310	20	258	145	2150
RCS-S	18	332	18	261	18	335	21	437	21	393	20	224	20	335	21	301	157	2618
RCS-T	16	274	18	260	16	314	20	296	13	160	20	256	21	372	21	277	145	2209
RCS-V	31	280	18	228	17	234	18	300	18	210	21	246	20	299	28	448	171	2245
RCS-W	18	280	18	286	18	303	21	278	21	333	20	382	20	375	20	320	156	2557
RCS-X	18	242	17	211	17	274	21	260	19	261	20	357	21	446	20	325	153	2376
Total	501	7468	489	6754	497	8364	524	8056	487	7393	531	7858	529	8384	549	7845	4107	62,122

Note: RCS stands for recombinant congenic strains. RCS-A… RCS-X refer to a series of 19 RCS derived from the inbred lines 6_3 and 7_2.

cantly higher than that of the progenitor lines 6_3 and 7_2 in Year 2005 to Year 2008 ($P < 0.01$) but comparable from Year 2006 to Year 2012 ($P > 0.05$). In contrast, the fertility of RCS-J was estimated with the lowest average fertility in 3 out of the 8 years (2005, 2010, and 2012), on the lower side in 3 of the 8 years (2007, 2009, and 2011), and at about the middle of the range in the other two years (2006 and 2008). The average fertilities of the progenitor lines 6_3 and 7_2 varied from year to year and were not significantly different from each other ($P > 0.05$) within any of the 8 years.

3.2. Embryo Mortality

Embryo mortality measures the percentage of embryos dead-in-shell during early period of incubation, also known as early embryonic mortality, which directly leads to reduction in hatchability of fertile eggs in poultry. Embryo mortality of all the inbred lines varied significantly during the years ($P < 0.0001$) with average embryo mortality of all the inbred lines ranged from 20.7% ± 0.6% in the Year 2012 to 35.2% ± 0.7% in Year 2011. The average embryo mortality of the eight years varied significantly among the inbred lines ($P < 0.0001$) with a range from 14.5% ± 0.7% for RCS-P up to 47.0% ± 1.2% for RCS-M. The average embryo mortality of the

Table 2. Fertility of 21 inbred experimental lines of White Leghorns.

Line	Estimated fertility (mean ± standard error) by year (%)								Average fertility
	2005	2006	2007	2008	2009	2010	2011	2012	
Line 6_3	79.8 ± 2.0^{de}	85.2 ± 1.7^{bcdef}	74.9 ± 1.7^{de}	67.5 ± 2.0^{h}	91.5 ± 1.3^{abc}	95.0 ± 0.9^{ab}	89.9 ± 1.1^{abc}	85.3 ± 1.3^{abcd}	82.4 ± 0.6^{fg}
Line 7_2	73.5 ± 2.5^{e}	85.7 ± 1.5^{bcdef}	67.7 ± 2.3^{ef}	73.3 ± 2.4^{gh}	87.8 ± 1.7^{abcd}	95.3 ± 1.0^{ab}	93.2 ± 1.2^{ab}	86.3 ± 1.9^{abcd}	81.6 ± 0.8^{g}
RCS-A	85.8 ± 2.8^{bcd}	80.5 ± 3.1^{efg}	94.3 ± 1.8^{a}	76.6 ± 3.1^{fg}	85.6 ± 3.7^{bcde}	94.4 ± 1.3^{abc}	80.0 ± 3.2^{def}	83.0 ± 2.3^{bcd}	84.8 ± 1.0^{ef}
RCS-B	92.1 ± 1.7^{abc}	92.8 ± 1.8^{abcd}	91.5 ± 1.9^{a}	94.5 ± 1.2^{abc}	91.6 ± 1.5^{abc}	98.5 ± 0.6^{a}	98.0 ± 1.0^{a}	90.9 ± 1.7^{ab}	93.6 ± 0.5^{ab}
RCS-C	94.3 ± 1.5^{ab}	88.7 ± 2.0^{abcde}	87.1 ± 2.8^{ab}	94.5 ± 1.3^{abc}	93.6 ± 1.3^{ab}	97.6 ± 0.9^{a}	96.1 ± 1.1^{ab}	81.8 ± 2.6^{bcd}	91.6 ± 0.7^{bc}
RCS-D	92.9 ± 1.8^{abc}	91.5 ± 2.2^{abcde}	91.8 ± 2.8^{a}	97.5 ± 1.0^{a}	94.0 ± 1.5^{ab}	96.3 ± 1.3^{ab}	94.7 ± 1.6^{ab}	84.8 ± 2.4^{abcd}	92.8 ± 0.7^{bc}
RCS-F	91.6 ± 2.0^{abc}	87.0 ± 2.6^{abcdef}	90.0 ± 2.3^{a}	85.5 ± 2.6^{bcdef}	86.0 ± 2.5^{bcde}	93.4 ± 1.8^{abc}	84.7 ± 3.1^{cde}	63.8 ± 3.4^{e}	84.3 ± 1.0^{ef}
RCS-G	95.3 ± 1.6^{ab}	96.6 ± 1.3^{ab}	94.8 ± 1.3^{a}	96.4 ± 1.1^{ab}	92.1 ± 2.5^{abc}	84.6 ± 2.8^{de}	93.5 ± 1.5^{ab}	76.7 ± 2.5^{d}	89.9 ± 0.8^{cd}
RCS-I	92.0 ± 2.3^{abc}	88.5 ± 2.3^{abcde}	79.7 ± 2.9^{bcd}	84.9 ± 3.2^{bcdef}	87.7 ± 1.8^{abcd}	93.2 ± 1.5^{abc}	93.9 ± 1.5^{ab}	78.1 ± 3.0^{cd}	86.8 ± 0.9^{de}
RCS-J	73.6 ± 3.3^{e}	86.8 ± 1.9^{abcdef}	77.1 ± 3.7^{cd}	84.7 ± 3.0^{bcdef}	79.6 ± 3.1^{de}	70.6 ± 3.2^{f}	77.6 ± 2.5^{ef}	49.4 ± 3.8^{f}	72.9 ± 1.3^{h}
RCS-K	88.3 ± 2.4^{abc}	87.6 ± 2.4^{abcde}	90.9 ± 2.3^{a}	92.6 ± 1.7^{abcd}	86.2 ± 2.2^{bcde}	94.5 ± 1.5^{abc}	88.4 ± 2.4^{bcd}	85.4 ± 2.5^{abcd}	89.1 ± 0.8^{cd}
RCS-L	92.0 ± 2.1^{abc}	83.7 ± 2.4^{cdef}	91.7 ± 1.8^{a}	89.9 ± 2.1^{abcde}	85.9 ± 2.2^{bcde}	94.7 ± 1.2^{abc}	81.5 ± 2.5^{cde}	88.2 ± 2.0^{abc}	88.5 ± 0.8^{cd}
RCS-M	90.3 ± 2.0^{abc}	94.6 ± 2.2^{abc}	94.4 ± 1.6^{a}	93.5 ± 1.4^{abcd}	92.5 ± 1.9^{abc}	95.0 ± 1.5^{ab}	93.3 ± 1.8^{ab}	92.1 ± 1.8^{ab}	93.0 ± 0.7^{ab}
RCS-N	91.0 ± 2.4^{abc}	89.2 ± 2.2^{abcde}	94.7 ± 1.2^{a}	82.5 ± 3.8^{cdefg}	71.9 ± 4.3^{f}	92.1 ± 1.9^{abc}	84.1 ± 3.4^{cde}	82.0 ± 3.8^{bcd}	85.9 ± 1.1^{de}
RCS-P	98.0 ± 0.9^{a}	97.8 ± 0.8^{a}	96.6 ± 1.1^{a}	98.4 ± 0.8^{a}	96.4 ± 1.1^{a}	98.1 ± 0.7^{a}	95.5 ± 1.2^{ab}	95.0 ± 1.6^{a}	96.8 ± 0.4^{a}
RCS-R	81.2 ± 3.2^{cd}	87.8 ± 3.6^{abcde}	89.3 ± 3.4^{ab}	70.5 ± 4.2^{h}	81.8 ± 3.0^{de}	90.7 ± 2.4^{bcd}	93.7 ± 1.5^{ab}	85.5 ± 2.5^{abcd}	84.4 ± 1.1^{ef}
RCS-S	87.9 ± 2.2^{abc}	72.6 ± 3.9^{g}	96.0 ± 1.1^{a}	90.2 ± 2.1^{abcde}	83.8 ± 2.7^{cde}	83.5 ± 3.8^{de}	90.1 ± 1.9^{abc}	69.5 ± 3.6^{e}	84.5 ± 1.0^{ef}
RCS-T	89.9 ± 2.2^{abc}	84.8 ± 3.4^{bcdef}	90.8 ± 1.9^{a}	82.6 ± 3.8^{cdefg}	84.2 ± 3.8^{cde}	80.7 ± 3.4^{e}	58.2 ± 5.5^{g}	89.3 ± 2.1^{abc}	82.6 ± 1.3^{fg}
RCS-V	87.4 ± 2.6^{abc}	77.0 ± 3.7^{fg}	73.1 ± 3.5^{de}	86.9 ± 2.2^{abcdef}	80.5 ± 3.0^{de}	74.8 ± 4.0^{f}	88.7 ± 2.6^{abc}	79.8 ± 2.5^{cd}	81.5 ± 1.1^{g}
RCS-W	87.5 ± 2.3^{abc}	89.0 ± 2.2^{abcde}	89.9 ± 2.2^{ab}	87.7 ± 3.6^{abcdef}	88.7 ± 2.1^{abcd}	90.2 ± 2.1^{bcd}	73.7 ± 3.1^{f}	69.0 ± 3.4^{e}	83.5 ± 1.1^{fg}
RCS-X	82.1 ± 3.1^{cd}	81.5 ± 2.9^{defg}	60.9 ± 5.6^{f}	81.5 ± 3.5^{cdefg}	81.4 ± 2.9^{de}	96.3 ± 1.1^{ab}	94.9 ± 1.3^{ab}	89.4 ± 2.3^{abc}	84.8 ± 1.1^{ef}

progenitor background line 6_3 was 28.3% ± 0.6%, which was significantly different from the 15.7% ± 0.6% average embryo mortality of the progenitor donor line 7_2 ($P < 0.01$). **Figure 2** graphically illustrates the relative rank of average embryo mortalities of the 21 inbred lines during the period of the eight years.

The estimates of the average embryo mortality for the 21 inbred experimental lines of chickens in each year are given in **Table 3**. Among the 21 inbred lines, RCS-M was ranked with the highest average embryo mortality in year 2005-2007 and year 2010-2012, followed by RCS-V with relatively high embryo mortality consistent throughout the 8 years ($P < 0.05$) in comparison to the other lines. In contrast, the RCS-K and RCS-P were consistently on the lowest or lower side of the rank in average embryo mortality from Year 2005 to Year 2012 ($P < 0.05$). The progenitor donor line 7_2 was observed with consistently lower embryo mortality percentages (12.1% - 20.6%) compared to the background line 6_3 (19.2% - 43.6%), and statistically differed in 4 (2006, 2008, 2009, and 2011) out the 8 years ($P < 0.05$, **Table 3**).

3.3. Hatchability of Fertile Eggs

Hatchability of fertile eggs measures the percentage of viable chicks hatched from fertile eggs within given numbers of eggs set in incubation. The average hatchability of fertile eggs of all the 21 inbred lines varied significantly during the eight years ($P < 0.0001$) with a range from 36.1% ± 0.8% in Year 2011 to 54.8.2% ± 0.8% in Year 2012. The average hatchability of fertile eggs during the eight year period also varied significantly among the inbred lines ($P < 0.0001$) with a range between 26.5% ± 1.1% for RCS-M and 66.8% ± 0.8% for the

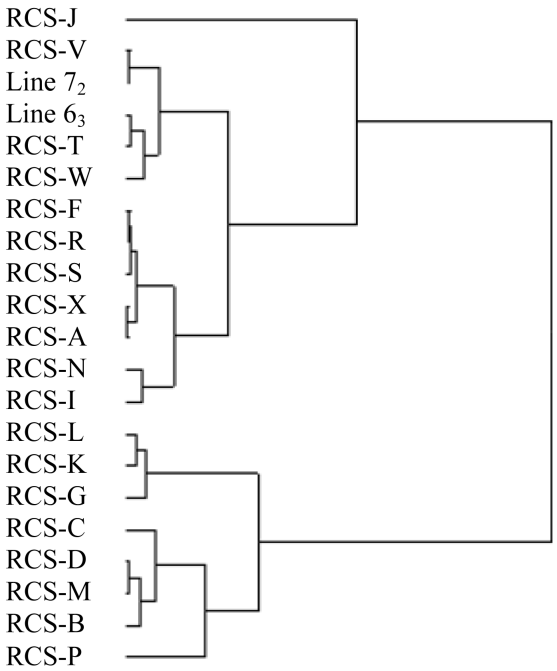

Figure 1. A hierarchical clustering tree depicts differences and similarities between the progenitor lines 6_3 and 7_2 and the recombinant congenic strains in fertility realized during a consecutive period of eight years (2005-2012). The progenitor lines 6_3 and 7_2 were similar to each other and were on the lower fertility side of the whole group (82.4% ± 0.60% and 81.6% ± 0.77%, respectively), similar to RCS-T, RCS-V, and RCS-W, and relatively distant to the rest of RCS in fertility. As shown, RCS-J had the lowest (72.9% ± 1.29%) and RCS-P had the highest (96.8% ± 0.41%) average fertility among the group during the years.

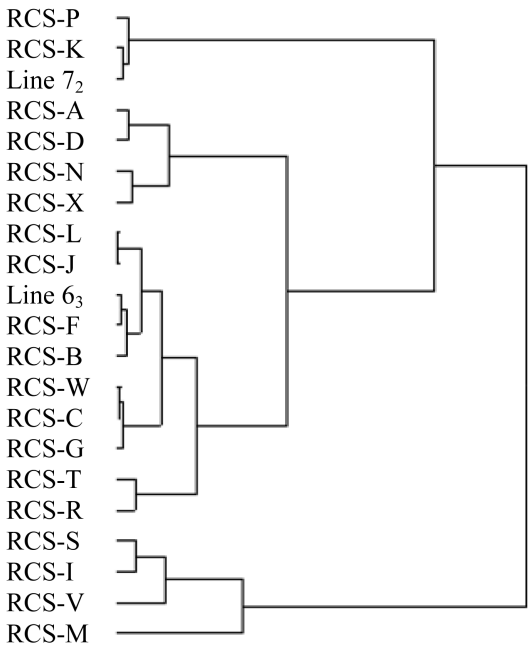

Figure 2. A hierarchical clustering tree depicts differences and similarities among the 21 lines in embryo mortality during the eight years. The progenitor donor line 7_2 had much lower average embryo mortality (15.7% ± 0.63%) than the background progenitor line 6_3 (28.3% ± 0.64%), similar with the RCS-P and RCS-K and relatively distant from the rest of RCS. RCS-P had the lowest and the RCS-M had the highest average embryo mortality (14.5% ± 0.71% and 47.0% ± 1.24%, respectively) during the years.

Table 3. Embryo mortality of 21 inbred experimental lines of White Leghorns.

Line	Estimated embryo mortality (mean ± standard error) by year (%)								Average embryo mortality
	2005	2006	2007	2008	2009	2010	2011	2012	
Line 6_3	23.8 ± 1.8^{cdef}	32.2 ± 2.0^{def}	25.0 ± 1.5^{def}	30.7 ± 1.8^{bcde}	25.8 ± 1.9^{efg}	27.4 ± 1.7^{cdef}	43.6 ± 1.8^{abcd}	19.2 ± 1.5^{efg}	28.3 ± 0.6^{def}
Line 7_2	16.4 ± 2.0^{fgh}	14.0 ± 1.7^{gh}	12.1 ± 1.8^{fg}	14.3 ± 1.8^{hi}	14.8 ± 1.7^{h}	20.6 ± 1.8^{ef}	16.7 ± 1.9^{g}	12.7 ± 1.6^{g}	15.7 ± 0.6^{h}
RCS-A	15.7 ± 2.6^{fgh}	13.2 ± 3.1^{h}	19.2 ± 2.6^{efg}	18.2 ± 3.4^{fghi}	33.5 ± 4.1^{cde}	28.0 ± 2.8^{bcde}	29.8 ± 3.4^{ef}	16.2 ± 2.3^{efg}	21.2 ± 1.1^{g}
RCS-B	21.1 ± 2.8^{cdef}	37.6 ± 3.5^{bcde}	20.0 ± 2.7^{efg}	24.6 ± 2.5^{efg}	40.1 ± 2.7^{bc}	32.7 ± 3.1^{bcde}	43.6 ± 3.4^{abcd}	14.6 ± 2.2^{fg}	29.2 ± 1.1^{def}
RCS-C	15.7 ± 2.6^{fgh}	45.9 ± 2.9^{abc}	25.4 ± 3.1^{def}	22.5 ± 2.7^{efgh}	36.2 ± 3.3^{bcd}	36.2 ± 3.0^{bc}	38.4 ± 3.3^{bcde}	23.3 ± 3.1^{bcdef}	30.1 ± 1.2^{de}
RCS-D	18.4 ± 3.1^{efg}	25.5 ± 3.5^{ef}	20.1 ± 3.4^{efg}	10.8 ± 2.0^{i}	24.7 ± 2.3^{efg}	18.8 ± 3.1^{f}	27.8 ± 3.5^{f}	28.9 ± 3.7^{bcd}	22.2 ± 1.1^{g}
RCS-F	35.3 ± 2.9^{ab}	48.3 ± 4.5^{abc}	22.8 ± 3.2^{efg}	26.3 ± 3.3^{def}	30.6 ± 3.0^{cdef}	25.7 ± 3.5^{def}	31.6 ± 3.3^{def}	17.6 ± 3.1^{efg}	28.6 ± 1.2^{def}
RCS-G	23.5 ± 2.8^{cdef}	40.6 ± 4.3^{bcd}	35.6 ± 2.9^{bc}	27.1 ± 3.8^{def}	34.7 ± 5.5^{cde}	29.1 ± 3.3^{bcde}	42.9 ± 3.7^{abcd}	22.5 ± 2.7^{cdef}	30.5 ± 1.3^{de}
RCS-I	29.5 ± 3.5^{bc}	41.3 ± 3.8^{bcd}	37.9 ± 3.9^{bc}	38.4 ± 4.0^{ab}	52.8 ± 3.6^{a}	36.1 ± 2.8^{bc}	42.1 ± 3.9^{bcde}	20.6 ± 3.1^{defg}	37.4 ± 1.3^{b}
RCS-J	23.8 ± 3.4^{cdef}	25.4 ± 3.4^{ef}	34.4 ± 3.9^{bc}	26.6 ± 3.7^{def}	20.9 ± 2.7^{fgh}	33.3 ± 3.9^{bcde}	32.3 ± 3.0^{def}	24.2 ± 3.9^{bcde}	27.4 ± 1.3^{efg}
RCS-K	8.6 ± 1.8^{h}	15.1 ± 2.8^{gh}	11.8 ± 2.5^{g}	10.5 ± 2.0^{i}	16.8 ± 2.6^{gh}	16.7 ± 2.2^{f}	26.9 ± 2.9^{f}	13.9 ± 2.3^{fg}	15.1 ± 0.9^{h}
RCS-L	15.0 ± 2.3^{fgh}	32.2 ± 3.9^{def}	38.2 ± 4.2^{bc}	23.3 ± 3.0^{efg}	31.7 ± 3.2^{cde}	30.2 ± 3.0^{bcde}	32.3 ± 3.2^{def}	19.7 ± 3.2^{efg}	27.3 ± 1.2^{efg}
RCS-M	40.4 ± 2.7^{a}	56.8 ± 4.2^{a}	57.0 ± 3.9^{a}	36.9 ± 3.5^{ab}	44.9 ± 3.3^{ab}	56.5 ± 3.2^{a}	54.7 ± 3.6^{a}	41.3 ± 2.9^{a}	47.0 ± 1.2^{a}
RCS-N	21.1 ± 2.5^{cdef}	33.9 ± 3.8^{cde}	20.0 ± 2.9^{efg}	15.2 ± 3.2^{h}	26.0 ± 4.6^{defg}	27.8 ± 4.1^{cdef}	40.9 ± 4.5^{bcde}	15.4 ± 3.8^{fg}	24.3 ± 1.3^{fg}
RCS-P	10.0 ± 1.6^{gh}	20.0 ± 2.4^{fg}	12.7 ± 1.7^{fg}	14.3 ± 2.1^{hi}	16.5 ± 2.1^{gh}	16.8 ± 2.2^{f}	16.6 ± 1.9^{g}	11.8 ± 1.9^{g}	14.5 ± 0.7^{h}
RCS-R	25.9 ± 3.7^{cde}	58.7 ± 5.1^{a}	20.1 ± 3.2^{efg}	43.0 ± 4.5^{a}	36.4 ± 3.5^{bc}	31.9 ± 4.0^{bcde}	30.7 ± 3.3^{def}	30.9 ± 3.7^{bc}	33.7 ± 1.4^{cd}
RCS-S	28.5 ± 3.0^{bcd}	43.6 ± 5.2^{bcd}	25.7 ± 3.8^{def}	44.3 ± 3.3^{a}	46.4 ± 3.2^{ab}	40.2 ± 4.5^{b}	34.0 ± 2.7^{cdef}	24.9 ± 3.0^{bcde}	35.7 ± 1.3^{bc}
RCS-T	18.4 ± 2.8^{efg}	47.1 ± 3.3^{abc}	26.3 ± 2.9^{cde}	37.0 ± 3.9^{ab}	51.8 ± 4.6^{a}	35.1 ± 3.6^{bcd}	42.3 ± 5.2^{bcde}	14.9 ± 2.5^{fg}	32.1 ± 1.4^{d}
RCS-V	38.8 ± 4.3^{ab}	53.2 ± 5.0^{ab}	47.6 ± 4.7^{ab}	35.6 ± 2.8^{abc}	46.2 ± 4.0^{ab}	37.0 ± 4.6^{bc}	46.9 ± 4.1^{ab}	31.8 ± 3.0^{b}	40.2 ± 1.4^{b}
RCS-W	22.4 ± 3.0^{cdef}	33.1 ± 3.0^{cde}	27.1 ± 3.3^{cde}	27.0 ± 3.6^{def}	37.5 ± 2.9^{bc}	30.8 ± 3.1^{bcde}	40.6 ± 4.0^{bcde}	20.4 ± 3.0^{defg}	29.9 ± 1.2^{de}
RCS-X	24.1 ± 3.4^{cdef}	41.3 ± 5.1^{bcd}	33.6 ± 5.0^{cde}	14.2 ± 3.4^{hi}	32.9 ± 3.9^{cde}	24.1 ± 2.8^{def}	29.1 ± 2.9^{ef}	14.5 ± 2.3^{fg}	25.6 ± 1.3^{fg}

donor line 7_2. The average hatchability of the progenitor background line 6_3 was 46.6% ± 0.8%, which was significantly lower than the progenitor donor line 7_2 ($P < 0.01$, **Table 4**). A hierarchical clustering tree was also constructed to graphically depict the relative average hatchability of the 21 inbred lines for the eight year period (**Figure 3**).

The estimates for hatchability of fertile eggs for each of the 21 inbred experimental lines of chickens from Year 2005 to Year 2012 are given in **Table 4**. The progenitor donor line 7_2 was ranked with the highest hatchability of fertile eggs in 6 (2005-2009, 2012) out of the 8 years, and was also among the highest hatchability group of lines in the other two years (2010, 2011). In contrast, the RCS-M was ranked as the line with the lowest hatchability in four years (2005, 2006, 2010, and 2012) and among the relatively low hatchability groups in the other four years (2007-2009, 2011). Some of the lines, including RCS-C, RCS-A and RCS-D, varied greatly in hatchability from year to year (**Table 4**).

4. Discussion

Inbred lines are invaluable resources for biological research, which provide experimental animals of high constancy within each of individual lines, and of distinctly genetic diversity between lines. The animals with such characteristics enable high detection power of biological experiments for difference in treatment and/or genetic effects on biological traits of interest over time and places with relatively high repeatability [10] [14] [18] [20] [23].

Table 4. Hatchability of 21 inbred experimental lines of White Leghorns.

Line	Estimated hatchability (mean ± standard error) by year (%)								Average hatchability
	2005	2006	2007	2008	2009	2010	2011	2012	
Line 6_3	54.9 ± 2.1^{bcd}	48.0 ± 2.2^{c}	44.5 ± 1.9^{bcd}	43.4 ± 2.2^{def}	50.7 ± 2.2^{b}	49.4 ± 2.1^{bcd}	29.4 ± 2.0^{gh}	54.5 ± 2.1^{c}	46.6 ± 0.8^{cde}
Line 7_2	71.5 ± 2.4^{a}	71.1 ± 2.0^{a}	67.8 ± 2.2^{a}	70.3 ± 2.4^{a}	63.2 ± 2.2^{a}	59.6 ± 2.3^{ab}	54.7 ± 2.7^{abc}	72.2 ± 2.2^{a}	66.8 ± 0.8^{a}
RCS-A	63.9 ± 3.3^{ab}	63.2 ± 4.3^{ab}	48.8 ± 5.8^{bc}	42.4 ± 5.1^{defg}	33.7 ± 5.1^{defg}	49.5 ± 3.8^{bcd}	36.4 ± 4.7^{defg}	68.4 ± 3.2^{ab}	52.3 ± 1.7^{bc}
RCS-B	64.1 ± 3.3^{ab}	46.9 ± 3.5^{bc}	44.6 ± 6.1^{bcd}	39.4 ± 4.4^{defg}	28.9 ± 3.7^{fgh}	55.8 ± 3.4^{b}	34.0 ± 3.4^{efgh}	70.4 ± 2.7^{a}	48.4 ± 1.5^{cd}
RCS-C	64.2 ± 3.3^{ab}	29.8 ± 2.7^{de}	36.9 ± 4.3^{cde}	48.5 ± 3.9^{bcde}	24.4 ± 2.8^{fghi}	35.5 ± 3.7^{efg}	30.5 ± 3.1^{fgh}	42.0 ± 3.6^{de}	39.0 ± 1.3^{fg}
RCS-D	68.2 ± 3.8^{a}	64.3 ± 3.8^{ab}	50.6 ± 6.0^{bc}	53.2 ± 5.4^{bcd}	30.1 ± 3.9^{efg}	68.9 ± 3.6^{a}	57.7 ± 3.8^{a}	53.3 ± 3.8^{c}	54.2 ± 1.6^{bc}
RCS-F	35.5 ± 3.1^{fgh}	29.9 ± 4.1^{def}	22.7 ± 4.1^{fg}	35.7 ± 4.2^{efg}	27.3 ± 3.4^{fgh}	53.0 ± 4.4^{bc}	35.4 ± 4.1^{efgh}	59.9 ± 3.8^{bc}	38.1 ± 1.5^{g}
RCS-G	39.5 ± 3.2^{fg}	22.2 ± 3.3^{f}	29.3 ± 3.3^{defg}	40.3 ± 3.6^{defg}	25.3 ± 4.8^{fghi}	38.9 ± 3.5^{def}	14.2 ± 2.6^{i}	38.0 ± 3.4^{ef}	32.4 ± 1.3^{h}
RCS-I	36.5 ± 3.6^{fgh}	24.6 ± 3.0^{ef}	22.0 ± 3.0^{fg}	36.4 ± 4.0^{efg}	16.5 ± 2.8^{i}	38.8 ± 3.6^{def}	27.5 ± 3.7^{fg}	49.2 ± 4.0^{cde}	31.5 ± 1.3^{h}
RCS-J	34.1 ± 3.8^{fgh}	43.8 ± 4.2^{c}	30.3 ± 3.6^{defg}	43.8 ± 4.2^{def}	35.2 ± 3.^{def}	38.3 ± 4.0^{def}	25.2 ± 3.1^{gh}	50.1 ± 4.4^{cde}	37.3 ± 1.4^{g}
RCS-K	53.9 ± 5.9^{bcd}	70.9 ± 3.6^{a}	45.4 ± 5.9^{bcd}	50.6 ± 5.0^{bcde}	41.5 ± 5.2^{bcd}	69.3 ± 2.9^{a}	56.7 ± 3.0^{ab}	71.4 ± 3.2^{a}	57.2 ± 1.7^{b}
RCS-L	51.9 ± 4.4^{cde}	39.8 ± 4.0^{cd}	37.8 ± 4.6^{cde}	34.2 ± 4.3^{efg}	36.9 ± 3.7^{cde}	40.6 ± 3.4^{def}	34.4 ± 3.5^{efgh}	51.1 ± 3.6^{cd}	41.0 ± 1.4^{efg}
RCS-M	27.4 ± 3.4^{h}	21.5 ± 3.5^{f}	18.9 ± 3.3^{fg}	32.9 ± 2.9^{fg}	22.4 ± 3.1^{hi}	25.8 ± 2.7^{g}	28.0 ± 3.4^{gh}	28.7 ± 2.9^{f}	26.5 ± 1.1^{i}
RCS-N	43.5 ± 4.5^{def}	30.4 ± 4.3^{def}	30.7 ± 4.0^{defg}	58.6 ± 4.3^{abc}	47.1 ± 5.2^{bc}	42.7 ± 4.1^{cde}	24.6 ± 3.4^{gh}	57.5 ± 4.4^{bc}	42.4 ± 1.7^{efg}
RCS-P	55.7 ± 3.6^{bc}	45.8 ± 3.2^{bc}	34.2 ± 4.4^{cdef}	61.8 ± 3.6^{ab}	27.1 ± 3.4^{fgh}	53.7 ± 3.1^{bc}	44.8 ± 2.7^{cde}	57.6 ± 3.3^{bc}	47.6 ± 1.3^{cd}
RCS-R	53.0 ± 4.3^{bcd}	24.7 ± 4.2^{ef}	49.9 ± 6.7^{bc}	45.9 ± 4.5^{def}	42.2 ± 3.8^{bc}	53.2 ± 4.0^{bc}	46.8 ± 4.1^{bcd}	55.4 ± 3.9^{c}	47.9 ± 1.6^{cd}
RCS-S	38.5 ± 3.2^{fgh}	29.2 ± 4.4^{de}	35.1 ± 4.3^{cde}	24.7 ± 2.7^{gh}	26.2 ± 3.1^{fgh}	30.6 ± 4.4^{fg}	27.8 ± 3.2^{gh}	42.4 ± 4.0^{de}	31.9 ± 1.3^{h}
RCS-T	64.3 ± 3.6^{ab}	31.1 ± 3.6^{de}	43.3 ± 3.0^{bcde}	38.2 ± 3.5^{defg}	23.9 ± 4.1^{ghi}	43.8 ± 4.6^{cde}	41.5 ± 5.4^{def}	53.5 ± 3.9^{c}	44.0 ± 1.5^{defg}
RCS-V	29.7 ± 3.4^{gh}	22.9 ± 4.4^{f}	16.3 ± 3.3^{g}	34.2 ± 3.0^{efg}	24.4 ± 3.7^{fghi}	34.3 ± 4.8^{efg}	24.1 ± 3.1^{h}	40.8 ± 3.1^{ef}	30.4 ± 1.3^{h}
RCS-W	40.7 ± 5.4^{efg}	49.3 ± 3.6^{bc}	33.2 ± 4.7^{cdef}	23.4 ± 4.7^{h}	36.3 ± 3.5^{cde}	49.1 ± 3.2^{bcd}	45.2 ± 3.8^{cde}	71.5 ± 3.2^{a}	45.0 ± 1.6^{cde}
RCS-X	41.6 ± 4.5^{efg}	26.2 ± 4.2^{ef}	25.6 ± 5.1^{efg}	47.3 ± 4.6^{cde}	24.4 ± 3.5^{fghi}	40.0 ± 3.1^{def}	30.8 ± 2.9^{fgh}	56.5 ± 3.4^{bc}	38.0 ± 1.5^{g}

ADOL at East Lansing, Michigan, currently maintains 35 special experimental lines of White Leghorns. Two-thirds of these lines are highly inbred. These lines are tested annually for purity by blood-typing tests using over 40 antisera. The breeders of all the lines are unique in that they are maintained in a quarantined state and, on the basis of frequent serologic tests for 11 pathogens, are considered free of infection from common poultry pathogens. Many of the lines each year produce either embryos or day-old chickens that are shipped out to meet the research or diagnosis needs of institutes or laboratories in the United States and around the world upon requests, in addition to fulfill the needs for research conducted on the site.

Line 6_3 and line 7_2 were initiated for establishment as inbred lines in 1939 at ADOL with hatching eggs from similar White Leghorn strains. Line 6_3 was one of the lines that were initiated to define genetic resistance to avian virus-induced tumors collectively termed as avian leucosis complex at the time. It was later shown by studies that the avian leucosis complex was consisted of tumors caused by two types of avian viruses commonly co-existed at the time in chicken houses, they are avian leucosis retroviruses (ALV) inducing principally lymphoid leukosis and Marek's disease herpesviruses (MDV) inducing MD in susceptible chickens [14] [20] [24] [25]. Line 6_3 chickens were selected for susceptibility to both ALV and MDV infection but uniquely resistance to tumor progression. Line 7_2 chickens were selected to define genetic resistance to subgroup A and B exogenous and subgroup E endogenous ALV infection but for susceptibility to MD tumors [14] [20].

A total of 19 RCS was set up for establishment as described by Bacon *et al.* [14] and Chang *et al.* [26]. Briefly, the series of RCS were developed by crossing the MD resistant line 6_3 and susceptible line 7_2 to produce the F_1

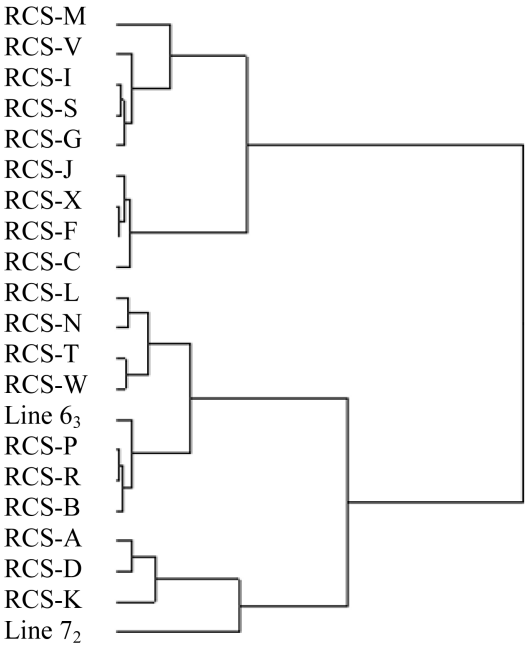

Figure 3. A hierarchical clustering tree depicts differences and similarities among the 21 lines in hatchability of the fertile eggs during the eight years. As illustrated, the progenitor line 7_2 achieved the highest average hatchability (66.8% ± 0.82%) during the years among all the lines and the line 6_3 failed close to the middle of the range (46.6% ± 0.76%). The lowest average hatchability was observed in the RCS-M (26.5% ± 1.14%) among all the lines over the years.

followed by two consecutive backcrosses to the background line 6_3. The series of RCS were established from full-sib mating of the backcross-2 progeny [14]. In theory, each of the RCS on average maintains 7/8 of the line 6_3 genome background and received a random sample of 1/8 of the donor line 7_2 genome. All lines, 6_3, 7_2, and RCS, are *MHCB**2 haplotype. The series of 19 RCS are the only extensive set of RCS under development in chickens or any other livestock species. The 19 RCS are under development to identify non-MHC genes that influence traits differing between the progenitor lines 6_3 and 7_2. The progenitor lines 6_3 and 7_2 differ in many ways, including resistance to avian virus-induced tumors [14] [26]-[28], protective efficacy in response to vaccination followed by Marek's disease virus challenge [26] [29] [30], behavior and behavior associated characteristics [31] [32], primary and secondary lymphoid organ sizes [33], and genetic and epigenetic differences [34]-[40]. This study showed the two lines 6_3 and 7_2 significantly differ in embryo mortality and hatchability of fertile eggs ($P > 0.01$). When a RCS differs in phenotype of a trait from the rest of RCS, a limited number of responsible non-MHC genes may be identified for further analysis. Each of the RCS is now over 90% inbred, and inbreeding increases each generation.

Fertility in chickens is reported with a very low heritability of about 0.05 - 0.10 [41] [42], suggesting the fertility of chicken eggs is heavily influenced by factors other than genetics. The fertility of layer-type of chickens in commercial populations is as high as 97% [43]. The progenitor lines 6_3 and 7_2 were observed with relatively lower average fertilities, 82.4% ± 0.6% and 81.5% ± 1.1%, respectively, over the eight years in comparison with either the commercial populations or some of the RCS. This can be in part attributable to the high degree of inbreeding of the lines. The fertility within each of the inbred lines did fluctuate from year to year in the eight years and the both of the year and the line effects on fertility were statistically significant ($P < 0.0001$). Yet three of the RCS (RCS-B, RCS-M, and RCS-P) maintained fertility consistently over 90% each year in the eight years and had 93.6% ± 0.5% 93.0% ± 0.7%, and 96.8% ± 0.4% average fertility over the year period, respectively (**Table 2**). Furthermore, although the progenitor lines 6_3 and 7_2 did not differ in fertility during the eight years evaluated, ten of the 19 RCS were observed with average fertility significantly higher than the progenitor lines ($P < 0.05$) during the eight year period, which strongly suggested the existence of a substantial polygenic component underlying the fertility phenotype despite the known fact that fertility is a trait of low heritability.

Embryo mortality is regarded as a direct fitness trait that could significantly reduce the efficiency of reproduction and increase the costs of production performance in poultry [44]. Like fertility, embryo mortality is also

characterized with relatively low heritability, reportedly ranging from 0.06 to 0.12 [5]. The two progenitor lines 6_3 and 7_2 significantly differed ($P < 0.01$) in embryo mortality with an eight year average of 28.3% ± 0.6% and 15.7% ± 0.6% embryo mortality, respectively. The donor line 7_2 was among the group of the lowest average embryo mortality and was significantly lower in average embryo mortality than 17 out of 19 RCS while the background line 6_3 was only significantly lower than 3 RCS ($P < 0.05$) over the eight year period (**Table 3**, **Figure 2**). The sizable range (14.5% ± 0.7% - 47.0% ± 1.2%) of the average embryo mortality of the inbred lines during the eight year period indicated that the embryo mortality ratios of the inbred lines of chickens followed a polygenic model of inheritance.

Hatchability of fertile eggs is a major component of reproductive fitness. Reported estimates of heritability for hatchability of fertile eggs fall into the low to moderate category [5] [41]. Industry performance target for hatchability is set for greater than 83% [45]. The average hatchability of fertile eggs in the eight year period for the 21 inbred experimental lines ranged from 26.5% ± 1.1% (RCS-M) to 66.8% ± 0.8% (line 7_2), significantly lower than the industrial target, which was highly likely, in a large part, resulted from high inbreeding, since inbreeding is known to have a seriously negative impact on reproductive characteristics including hatchability in chickens [8]. The progenitor donor line 7_2 was one of the lines with the observed lowest average embryo mortality but was the line with the observed highest average hatchability of fertile eggs over the eight year period and significantly different from the background line 6_3 (**Table 3** & **Table 4**, $P < 0.01$). The large range of hatchability of fertile eggs among the RCS indicated this trait also follow a model of polygenic inheritance.

Earlier studies showed genetic background due to MHC haplotype differences does exert significant effects on production and reproduction characteristics in White Leghorns [18] [46]. The fact that the year to year effect on fertility, embryonic mortality, and hatchability of fertile eggs was very statistically significant ($P < 0.0001$) suggesting that environmental effects and random variations highly likely predominate. However, since all of the 21 inbred lines of chickens in this study were housed and managed under standardized control conditions on a specific pathogen free farm and all of the lines share the same MHC *B**2 haplotype, it should be within reason to assume that the differences in these traits within each year attributable to environmental influence were moderate to minimal. Thus, the statistically significant differences in fertility, embryo mortality, and hatchability of fertile eggs among the inbred lines strongly suggested that polygenic effects play a substantial role influencing the phenotypes of the reproduction characteristics of the inbred lines and the polygenic effects were imposed by non-MHC genes. Furthermore, both of the progenitor lines 6_3 and 7_2 averaged with relatively low fertility in contrast to over half of the RCS, which suggested multiple loci of genes positively affecting fertility are reciprocally fixed in the two lines, respectively. This speculated model for fertility, to some extent, would also be applicable for interpreting the progenitor lines' ranking positions with the RCS for embryonic mortality. On the contrary, the progenitor line 7_2 was ranked on the very top for hatchability of fertile eggs, and the line 6_3, at the middle, which indicated both of the progenitor lines 6_3 and 7_2 are likely oppositely fixed at multiple loci of genes negatively impacting on hatchability of fertile eggs, in addition to fixations at loci that positively affect hatchability, especially in the line 7_2. Findings from this study paves the way for further investigation on genetic and environmental influence over reproductive performance of inbred lines of chickens, and particularly in understanding and improving the reproduction fitness of invaluable genetic resources like these inbred lines.

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