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Genetics of male reproductive performance in White Leghorns

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Abstract

The ability to produce viable progeny is a complex trait, involving both male and female components. In poultry, mating ratios are usually 1 male to 6 to 12 females. Consequently, the impact of male reproductive failure is much greater than that for a female. In this study, the genetic determination of male reproductive performance, by natural mating and artificial insemination (AI), was evaluated. Semen quality was studied in 1,575 pre-selected (using a selection index of multiple egg production and quality traits) White Leghorn males of a single pure line from multiple generations. A subset of individuals with satisfactory semen quality (based on sperm count and motility) were further tested for subsequent fertility and hatchability. Genetic parameters for fertility (FER), hatch of fertile (HOF), hatch of set (HOS), sperm motility (SM), sperm count (SC), and fertility using AI (FER-AI) were estimated using single- and multi-trait animal models, with generation as fixed effect. Selected birds were genotyped using the 600K Affymetrix SNP chip. Genomic data were analyzed with the BayesB method. FER, HOS, and HOF were highly correlated, both genetically (0.82 to 0.99) and phenotypically (0.28 to 0.99), but genetic correlations with semen quality traits were not strong (0.05 to 0.43) and phenotypic correlations varied between generations (–0.13 to 0.14). Birds used for fertility and hatchability tests were pre-selected based on SM and SC, which could contribute to the lack of strong correlations between these traits (due to truncation of the distribution). Based on pedigree information, low to moderate heritabilities were estimated for reproductive traits (0.08 to 0.21). Markers explained a low proportion of phenotypic variance (0.04 to 0.15), probably due to stringent selection of genotyped individuals and the limited training set size. No genes with large effects were identified. Genomic estimated breeding values were more accurate than pedigree-based estimates but only for HOF and FERT-AI. Despite low estimates of accuracy in validation, genetic trends were positive for all analyzed traits. In conclusion, continued long-term selection can result in genetic improvement of reproductive performance of roosters.

Keywords

fertility, hatchability, semen quality, Leghorn male, genetics

Disciplines

Agriculture | Animal Sciences | Genetics and Genomics | Poultry or Avian Science

Comments

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GENETICS AND GENOMICS

Genetics of male reproductive performance in White Leghorns

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ABSTRACT The ability to produce viable progeny is a complex trait, involving both male and female components. In poultry, mating ratios are usually 1 male to 6 to 12 females. Consequently, the impact of male reproductive failure is much greater than that for a female. In this study, the genetic determination of male reproductive performance, by natural mating and artificial insemination (AI), was evaluated. Semen quality was studied in 1,575 pre-selected (using a selection index of multiple egg production and quality traits) White Leghorn males of a single pure line from multiple generations. A subset of individuals with satisfactory semen quality (based on sperm count and motility) were further tested for subsequent fertility and hatchability. Genetic parameters for fertility (FER), hatch of fertile (HOF), hatch of set (HOS), sperm motility (SM), sperm count (SC), and fertility using AI (FER-AI) were estimated using single- and multi-trait animal models, with generation as fixed effect. Selected birds were genotyped using the 600K Affymetrix SNP chip. Genomic data were analyzed with the BayesB method. FER, HOS, and HOF were highly correlated, both genetically (0.82 to 0.99) and phenotypically (0.28 to 0.99), but genetic correlations with semen quality traits were not strong (0.05 to 0.43) and phenotypic correlations varied between generations (–0.13 to 0.14). Birds used for fertility and hatchability tests were pre-selected based on SM and SC, which could contribute to the lack of strong correlations between these traits (due to truncation of the distribution). Based on pedigree information, low to moderate heritabilities were estimated for reproductive traits (0.08 to 0.21). Markers explained a low proportion of phenotypic variance (0.04 to 0.15), probably due to stringent selection of genotyped individuals and the limited training set size. No genes with large effects were identified. Genomic estimated breeding values were more accurate than pedigree-based estimates but only for HOF and FERT-AI. Despite low estimates of accuracy in validation, genetic trends were positive for all analyzed traits. In conclusion, continued long-term selection can result in genetic improvement of reproductive performance of roosters.

Key words: fertility, hatchability, semen quality, Leghorn male, genetics

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INTRODUCTION

The ability to produce viable progeny is a complex trait that involves genetic components of both male and female mating behavior, fertility, female potential to lay eggs, egg size and shape, eggshell quality, and embryo survival. Also, in the case of using artificial insemination (**AI**), human intervention further increases the complexity of the reproductive process by adding external factors such as semen collection, processing and storage, exposure to environmental factors (temperature, moisture, dust, etc.), and semen application to a receptive hen (dose, timing, and technique). Some of these traits have been shown to have a genetic component (Sapp et al., 2004; Wolc et al., 2010; Cavero et al., 2011) and some traits related to sperm quality have been shown to be predictive for male fertility (Froman et al., 1999) and were estimated to have higher heritability than direct measurements of reproductive performance (Soller et al., 1965; Bongalhardo et al., 2000, Hu et al., 2013a). However, there is limited literature describing genetic relationships between semen quality traits and reproductive traits. Froman and Rhoades (2013) published preliminary results which suggested that roosters with extreme phenotypes for sperm mobility differ in allele frequencies in several genomic regions, including the Z chromosome, but no QTL have been reported in the animalgenome.org database (Hu et al., 2013b) for chicken reproduction. Fertility and hatchability traits can be assigned as male and/or female traits but the study herein focused only in the male component.

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In this study, we quantified male reproductive performance of a White Leghorn line using 3 common reproductive performance parameters: the average percentage of eggs that were fertilized, the proportion of chicks hatched from the number of eggs set for incubation (hatch of set eggs, **HOS)**, and the proportion of chicks hatched based on the total number of fertile eggs (hatch of fertile eggs, **HOF**). Sperm quality was evaluated as sperm count (**SC**) and sperm motility (**SM**) using the Chicken Mobility Analyzer Accudenze, a device that measures sperm mobility by quantifying the mobile subpopulation of sperm within an ejaculate that penetrates a dense non-ionic and biologically inert solution (Froman and McLean, 1996). Male fertilizing ability was assessed following artificial insemination (**FER-AI**). The goal of this study was to estimate genetic parameters using pedigree and genomic data, and to identify regions in the genome that explain a substantial proportion of genetic variance for each of these traits.

MATERIAL AND METHODS

Phenotypes

Males were pre-selected on a selection index of pedigree-based BLUP and/or genomic estimated breeding value (**EBV**) for multiple production and quality traits measured in the female relatives, then tested for specific semen quality traits and behavior and reproductive performance with natural mating in floor pens. Sperm motility and count were evaluated from a single dose by the Chicken Mobility Analyzer Accudenz, which measures the size of a sperm subpopulation that penetrates a solution (Froman and McLean, 1996). Accudenz is a non-ionic, biologically inert, low molecular weight solute. The number of sperm that penetrate the Accudenz layer is estimated by measuring absorbance at 550 nm after a 5-min incubation at body temperature (41°C). Sperm mobility is obtained by quantifying the mobile subpopulation of sperm within an ejaculate (Froman and McLean, 1996). The 3 male fertility traits that were analyzed were collected from hatching eggs obtained from natural mating in stage 3 of male selection. Males (approximately 36 wk of age) were housed in single male floor pens with about 18 hens per male. Fertility (**FER**) was determined by standard candling while transferring the eggs to the hatcher. A fertile egg appeared as non-clear at candling, while clear eggs were recorded as infertile (actually a combination of infertile, dead germs and early embryo mortality), and the results were expressed as percentages. Hatch of set (**HOS)** was defined as the proportion of good quality viable chicks at hatch out of the total number of eggs that were set, and hatch of fertile (**HOF**) was the proportion of good quality viable chicks at hatch out of the total number of fertile eggs. On average, 479 eggs were set per male (range of 86 to 782). After the final stage of selection in each of 7 generations, semen was obtained from selected males and used for AI in pedigree matings, which provided additional records for fertility. Floor pen data of 1,239 males from 9 generations and sperm quality data from 1,575 males from 6 generations were used for variance component estimation. The data are summarized in Table 1. Pedigree including 143,519 individuals from 10 generations was used for all pedigree-based analyses. Birds were handled using standard breeding program procedures, according to the company's animal welfare policy approved by the veterinarian on staff.

Table 1. Summary of the data for male fertility.

	N	Average	SD	Min	Max	Median	CV
No_PEN MATES	1,239	15.5	3.12	10	18	18	20.15
EGGS_SET	1,239	479.4	183.70	86	782	521	38.32
FER (%)	1,239	74.8	18.66	0	98.2	79	24.96
HOF (%)	1,239	82.6	17.59	0	100	88.9	21.29
HOS (%)	1,239	64.7	19.62	0	94	69	30.31
SM	1,575	82.1	15.30	0	98	87	18.65
SC	1,575	8.8	2.32	0	14	9	26.35
FER_AI (%)	600	82.3	6.16	41.66	97	83	7.48

No_PEN MATES- number of hens per pen in the fertility and hatchability test, **EGGS_SET**- number of eggs set for incubation for the fertility and hatchability test, **FER**- percentage of fertilized eggs, **HOS**- the proportion of hatched chicks from the number of eggs set for incubation, **HOF**- the proportion of chicks hatched of the fertile eggs, **SC**- sperm count**, SM**- sperm motility**, FER_AI**- percentage of fertilized eggs using artificial insemination

Genotype Data

Only males that were selected in the final stage and contributed progeny were genotyped using the 600K Affymetrix panel (Kranis et al., 2013). From the 600K Affymetrix panel, 153,797 high-quality ("Recommended category" according to the Axiom Genotyping Solution Data analysis guide) and segregating SNPs were retained for 822 males from 7 generations. The genomic analyses were performed using the data of genotyped animals only.

Statistical Analyses

Genetic parameters were estimated with multi-trait animal models in ASReml (Gilmour et al., 2008), with generation as the only fixed effect. Because sperm quality data were not available for the early generations, 2 separate models were run. Genetic correlations among FER, HOF, HOS, SM, SC, and FER_AI were based on a 6-trait model, using 6 generations of data, except for genetic correlations between FER, HOS, and HOF, which were based on a 3-trait model that included all generations. Genetic trends were calculated based on the average pedigree-based EBV of all animals by generation.

Genome wide association analysis was performed for each of the trait above separately using the GenSel software (Garrick and Fernando, 2013). Phenotypic values were used as response variable and generation effect was fitted as a fixed effect in addition to random marker effects. The BayesB model, assuming that only 1% of

Table 2. Range of phenotypic correlations between the traits within a generation (above the diagonal) and phenotypic correlations across generations (below diagonal).

	FER	HOF	HOS	SM	SC	FER_AI
FER		0.28 to 0.94	0.63 to 0.99	−0.13 to 0.06	−0.03 to 0.16	−0.10 to 0.31
HOF	0.64		0.62 to 0.99	−0.08 to 0.12	−0.14 to 0.13	−0.11 to 0.13
HOS	0.92	0.8		−0.13 to 0.08	−0.12 to 0.13	−0.13 to 0.18
SM	0.21	0.01	0.16		−0.08 to 0.14	−0.03 to 0.09
SC	−0.05	−0.09	−0.11	0		−0.02 to 0.14

Table 3. Estimates of heritability (on diagonal), genetic (above diagonal), and residual (below diagonal) correlations; proportion of variance explained by markers (h^2_m), pedigree based accuracy (acc_{ped}), and marker based accuracy (acc_m).

	FER	HOF	HOS	SM	SC	FER_AI
FER	**0.21 ± 0.06**	0.82 ± 0.11	0.99 ± 0.02	0.43 ± 0.28	0.12 ± 0.24	−0.26 ± 0.30
HOF	0.68 ± 0.03	**0.13 ± 0.05**	0.88 ± 0.07	0.35 ± 0.31	0.05 ± 0.27	−0.05 ± 0.31
HOS	0.90 ± 0.01	0.83 ± 0.01	**0.20 ± 0.06**	0.39 ± 0.28	0.06 ± 0.24	−0.19 ± 0.28
SM	−0.05 ± 0.05	−0.01 ± 0.05	−0.05 ± 0.06	**0.08 ± 0.04**	0.56 ± 0.24	0.18 ± 0.32
SC	0.05 ± 0.06	0.05 ± 0.05	0.05 ± 0.06	0.07 ± 0.04	**0.13 ± 0.04**	0.49 ± 0.26
FER_AI	0.36 ± 0.07	0.20 ± 0.07	0.23 ± 0.08	0.03 ± 0.06	0.00 ± 0.06	**0.18 ± 0.08**
h^2_m	0.08	0.03	0.08	0. 04	0.14	0.15
acc_{ped}	0.04	0.25	0.07	−0.38	0.32	0.08
acc_m	−0.07	0.55	0.08	−0.22	0.38	0.62

Diagonal in bold are estimates of heritability.

SNPs is a priori associated with each trait, was used. The choice of 1% SNP was consistent with one of the study's goals of identifying genomic regions explaining a substantial proportion of genetic variance for the target trait. Genomic predictions were calculated as a sum of a product of allele counts and estimated allele effects across all SNPs. Accuracy was calculated as the correlation between EBV and phenotype in the last generation of the data, which was removed from training as the validation set, divided by the square root of pedigree-based heritability. Validation phenotypes were not adjusted for any effects because there were no identifiable fixed effects within generation. To keep the results comparable, pedigree-based EBV for accuracy comparisons were obtained from a single trait model.

RESULTS AND DISCUSSION

Phenotypic correlations across all generations and the minimum and maximum correlations from within generation analyses are in Table 2. There was large variation between the tests in the estimates of correlations between some of the traits. As expected, FER HOF and HOS were highly correlated at both the genetic and phenotypic levels, which confirms previous studies, for example by Cavero et al., (2011) and Savegnago et al., (2011). Fertility in floor pen mating was only weakly correlated with fertility using AI (r_p = –0.10 to 0.31), and also the phenotypic correlations between sperm quality and fertility were not very strong and ranged from slightly negative to slightly positive across generations (r_p = 0.03 to 0.14). Slightly negative estimates of genetic correlations between fertility in floor pens and under AI (r_G = –0.26) suggest that these may be 2 different genetic traits. The amount of data was limited and only a small proportion of variance was genetic; thus, the standard errors of genetic correlations were large. Natural mating behavior adds complexity to achieving fertility (Cook et al, 1972; Siegel, 1984) and also human intervention (including semen handling, dilution, use of extenders, exposure to environment moisture, dust, etc.) affects the volume of semen and other factors in the process of AI.

Estimates of heritability for fertility and hatchability (h^2 = 0.13 to 0.21) were similar to those previously reported (Sapp et al., 2004; Wolc et al., 2009, 2010). However, estimates of heritability of sperm motility (h^2 = 0.08) and count (h^2 = 0.13) were lower than reported in previous studies with different chicken breeds (Soller et al., 1965; Kabir et al., 2007; Hu et al., 2013a) but close to the results obtained in another White Leghorn population (Bongalhardo et al., 2000).

Estimates of the proportion of variance explained by markers were very low (h_m^2 = 0.03 to 0.15) for the genomic analyses (Table 3) and lower than the pedigree based estimates of heritability, except for SC. The lost heritability could be explained in part by data structure, as genomic data were collected only on sires that contributed to the next generation, thus animals with low fertility were not included and a large proportion of variation was not present when data was limited to genotyped animals. No regions with large effects were detected. Windows that explained the largest proportion of genetic variance were located on chromosome 1 at 192 Mb for FER, 0.98% of genetic variance; on chromosome 21 at 5 Mb for HOF, 0.7% of genetic variance; on chromosome 14 at 12 Mb for HOS, 1.1% of genetic variance; on chromosome 1 at 179 Mb for SC, 1.2% of genetic variance; on chromosome 21 at 5 Mb for SM, 0.9% of genetic variance; and on chromosome 13 at 8 Mb for FER_AI, 0.9% of genetic variance. The QTL

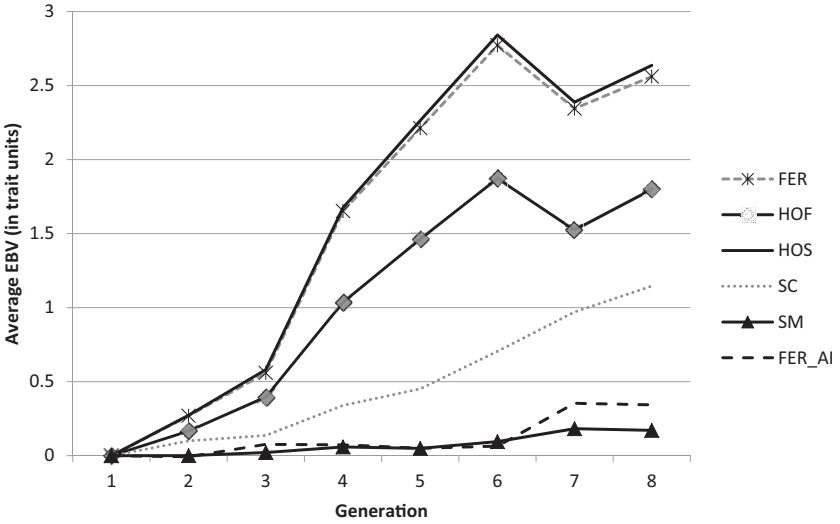

Figure 1. Genetic trends for reproductive traits in a White Leghorn Line. Average standard error of the mean was 0.015, 0.010, 0.015, 0.007, 0.002, 0.005 for FER, HOF, HOS, SPERM, MOB, SPERM, COUNT, FERAI, respectively.

database (www.animalgenome.org, Hu et al., 2013b) does not list any results for traits analyzed in this study, but Xu et al. (2010) reported a QTL for broodiness at a similar location on chromosome 13, which may share some hormonal regulation with the reproductive traits studied here. A similar location on chromosome 13 was also reported to contain a QTL for age at sexual maturity (Liu et al., 2011; Podisi et al., 2011).

The predictive ability of the pedigree-based EBV was low and marker information brought substantial improvement over pedigree but only for 2 (HOF and FER_AI) of the 6 analyzed traits. It must be stressed, however, that genotypes were available only for highly selected individuals, which results in underestimation of the accuracy of EBV (Edel et al., 2012). Despite the low accuracy in validation, genetic trends over 9 generations (Figure 1) were positive for all traits, indicating some improvement as a result of selection

In conclusion, reproductive performance of white leghorn males in the study herein has a limited genetic component that can be captured by pedigree and markers. No QTL of large effects were detected. Low heritability and slow response to selection were confirmed, as expected for fitness-related traits.

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REFERENCES

Axiom™ Genotyping Solution. Data analysis guide. https://assets.thermofisher.com/TFS-Assets/LSG/manuals/axiom_genotyping_solution_analysis_guide.pdf. Accessed on Oct. 2018.

Bongalhardo, D., N. Dionello, and M. Ledur. 2000. Parâmetros genéticos para caracteres de sêmen de aves White Leghorn.: 1. Herdabilidades e correlações. BMC Genet. 29:1320–1326.

Cavero, D., M. Schmutz, W. Icken, and R. Preisinger. 2011. Improving hatchability in white egg layer strains through breeding. Lohmann Info. 46:44–54.

Cook, W. T., P. B. Siegel, and K. Hinkelmann. 1972. Genetic analyses of male mating behavior in chickens. II. Crosses among selected and control lines. Behav. Genet. 2:289–300.

Edel, C., S. Neuner, R. Emmerling, and K. U. Götz. 2012. A note on using 'forward prediction' to assess precision and bias of genomic predictions. Interbull Bulletin. 46:16

Froman, D. P., A. J. Feltmann, M. L. Rhoads, and J. D. Kirby. 1999. Sperm mobility: a primary determinant of fertility in the domestic fowl (Gallus domesticus). Biol. Reprod. 61:400–405.

Froman, D. P., and D. J. McLean. 1996. Objective measurement of sperm motility based upon sperm penetration of Accudenz(R). Poult. Sci. 75:776–784.

Froman, D. P., and D. D. Rhoads. 2013. Breeding and genetics symposium: a systems biology definition for chicken semen quality. J. Anim. Sci. 91:523–529.

Garrick, D. J., and R. L. Fernando. 2013. Implementing a QTL detection study (GWAS) using genomic prediction methodology. Pages 275–298 in Genome-Wide Association Studies and Genomic Prediction, Methods in Molecular Biology, Vol. 1019. C. Gondro, J. van der Werf, and B. Hayes eds. Springer Science+Business Media, New York.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2008. ASReml User Guide Release 3.0. VSN Int. Ltd., Hemel Hempstead, UK.

Hu, J., J. L. Chen, J. Wen, G. P. Zhao, M. Q. Zheng, R. R. Liu, W. P. Liu, L. H. Zhao, G. F. Liu, and Z. W. Wang. 2013a. Estimation of the genetic parameters of semen quality in Beijing-You chickens. Poult. Sci. 92:2606–2612.

Hu, Z. L., C. A. Park, Z. L. Wu, and J. M. Reecy. 2013b. Animal QTLdb: an improved database tool for livestock animal QTL/association data dissemination in the post-genome era/association data dissemination in the post-genome era. Nucleic Acids Res. 41:D871–D879.

Kabir, M., O. O. Oni, and G. N. Akpa. 2007. Osborne selection index and semen traits interrelationships in Rhode Island Red and White Breeder cocks. Nucleic Acids Res. 6:999–1002.

Kranis, A., A. A. Gheyas, C. Boschiero, F. Turner, L. Yu, S. Smith, R. Talbot, and A. Pirani et al. 2013. Development of a high density 600K SNP genotyping array for chicken. BMC Genomics 14:59.

Liu, W., D. Li, J. Liu, S. Chen, L. Qu, J. Zheng, G. Xu, and N. Yang. 2011. A genome-wide SNP scan reveals novel loci for egg production and quality traits in white leghorn and brown-egg dwarf layers. PLoS One. 6:e28600.

Podisi, B. K., S. A. Knott, I. C. Dunn, A. S. Law, D. W. Burt, and P. M. Hocking. 2011. Overlap of quantitative trait loci for early growth rate, and for body weight and age at onset of sexual maturity in chickens. Reproduction 3:381–389.

Sapp, R. L., R. Rekaya, I. Misztal, and T. Wing. 2004. Male and female fertility and hatchability in chickens: a longitudinal mixed model approach. Poult. Sci. 83:1253–1259.

Savegnago, R. P., M. E. Buzanskas, B. N. Nunes, S. B. Ramos, M. C. Ledur, K. Nones, and D. P. Munari. 2011. Heritabilities and genetic correlations for reproductive traits in an F2 reciprocal cross chicken population. Genet. Mol. Res. 10:1247–1254.

Siegel, P. B. 1984. The role of behavior in poultry production: a review of research. Appl. Anim. Ethol. 11:299–316.

Soller, M., N. Snapir, and H. Schindler. 1965. Heritability of semen quantity, concentration and motility in White Rock roosters, and their genetic correlation with rate of gain. Poult. Sci. 44:1527–1529.

Wolc, A., I. M. S. White, V. Olori, and W. G. Hill. 2009. Inheritance of fertility in broiler chickens. Genet. Sel. Evol. 41:47.

Wolc, A., I. M. S. White, V. Olori, and W. G. Hill. 2010. Inheritance of hatchability in broiler chickens and its relationship to egg quality traits. Poult. Sci. 89:2334–2340.

Xu, H., X. Shen, M. Zhou, M. Fang, H. Zeng, Q. Nie, and X. Zhang. 2010. The genetic effects of the dopamine D1 receptor gene on chicken egg production and broodiness traits. BMC Genet. 11:17.