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Research Note: Phenotypic trends for the multigenerational advanced intercross of the Virginia body weight lines of chickens

P. B. Siegel,[*,1] C. F. Honaker,[*] and L. Andersson [†,‡]

[*]School of Animal Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA; [†]Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Uppsala University, S-75123 Uppsala, Sweden; and [‡]Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843, USA

ABSTRACT Random samples from generation S_{41} of the Virginia high and low 8-week body weight lines formed the base population for producing a multigenerational reciprocal intercross population. Although genetic mapping from this intercross has been reported, lacking are phenotypic trends across multiple generations. Here, we provide phenotypic information for the parental base population, the F_1 reciprocal cross, and subsequent segregating recombinant generations F_2 to F_{17}. Heterosis for the selected trait in the F_1 was negative for both reciprocal crosses. Phenotypic correlations for the selected trait in the recombinant generations were essentially nil for both males and females as was percent sexual dimorphism and coefficients of variation.

Key words: body weight, chicken, selection, intercross

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INTRODUCTION

The use of F_2 intercrosses is the method of choice for quantitative trait locus (**QTL**) mapping when genetic differences occur between divergent populations rather than as variation within outbred populations. However, a problem with this experimental design is the lack of recombination events in a single generation, which results in poor map resolution for QTLs in the F_2 generation. Advanced Intercross Lines (**AILs**) were thus proposed as a strategy to handle this problem. Darvasi and Soller (1995) wrote "an advanced intercross line…can provide more accurate estimates of QTL map locations than conventional mapping populations." This is because via an AIL, recombination events accumulate by generations, and thus mapping resolution will be enhanced. Two decades later, Gonzales and Palmer (2014) pointed out in their review that AILs maintained as outbred populations further reduced linkage disequilibrium between adjacent markers, thus allowing for finer scaled mapping.

Among farm animals, multigenerational AILs lends itself to chickens because of the short generation interval. The use of intercross populations for genetic mapping of numerous traits in chickens has thus become common (e.g., Jennen et al., 2005; Hasenstein and Lamont, 2007; Heifetz et al., 2009; Prashar et al., 2009; Redmond et al., 2011; Wang et al., 2020), including an especially long-term (F_{18}, F_{19}) one (van Goor et al., 2015) and the one described in this submission based on an intercross between the Virginia high and low weight selection lines (Jacobsson et al., 2005). The initial QTL mapping using the F_2 generation suggested that the selection response was highly polygenic with many loci each with a small phenotypic effect (Carlborg et al. 2006; Wahlberg et al., 2009). Genetic analysis using the AIL derived from this intercross confirmed this conclusion, enhanced the map resolution substantially, and demonstrated how epistatic interaction contributed to genetic variance (Pettersson et al., 2011; Sheng et al., 2015; Brandt et al., 2017; Zan et al. 2017; Lillie et al., 2018; Rönneburg et al., 2022). Our series of publications demonstrates the value of AIL as a resource for genetic analysis due to the enhanced mapping resolution. Both Darvasi and Soller (1995) and Gonzales and Palmer (2014) pointed out the "real world" issues of population size, relatedness among individuals, and breeding strategies when designing an AIL. A detailed example of these issues was provided by Heifetz et al. (2009), who focused on Marek's Disease.

Surprisingly, however, there is a general void in the AIL poultry literature on phenotypic changes in their mapping populations. Here we describe the design and phenotypic characteristics across 17 generations in the AIL we developed from the Virginia lines that had undergone long-term selection for 8-week body weight (Harrison et al., 2023).

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[1]Corresponding author: pbsiegel@vt.edu

MATERIALS AND METHODS

Animal Use and Care

All procedures and protocols used in this experiment were approved by the Institutional Animal Care and Use Committee at Virginia Tech.

Data

The base populations for the Virginia advanced intercross line (**AIL**) were the Virginia high (**HWS**) and low (**LWS**) lines. These lines, established from a common founder population, had undergone divergent selection for the single trait body weight at 8 weeks of age (Siegel, 1962; Harrison et al., 2023). In generation S_{41}, reciprocal crosses were made from individuals randomly selected from each line to produce an F_1.

The F_1 progeny were from matings of 10 HWS males with 22 LWS females, and 8 LWS males with 19 HWS females. The F_2 was from 8 males (4 LH and 4 HL males to 39 HL and 37 LH females). The F_3 was produced from 58 matings each from 1 male to 2 females. For the F_4, there were 3 matings of 1 male to 3 females, 47 of 1 to 2, and 32 of 1 to 1. Thereafter, all generations were by single pair matings ranging from 25 (F_{13}) to 57 (F_5). All matings were by artificial insemination with sire-dam assignments random with the restriction that none involved sibs.

Body weights (g) were obtained for 384 males and 413 females in the F_2 generation. Respective numbers for F_3 through F_{17} were 1,763 and 1,890. The age of breeders varied across generations because we were focused on accelerating the production of the AIL. Also, there were ongoing peripheral experiments with the AIL which influenced timing of the next generation. Thus, we expected a generational effect on 8 wk body weight which would not be unlike that seen for hatch effects that are routine for flocks over the course of their production cycle.

Husbandry

At hatch, chicks were wing banded for individual identification and vaccinated for Marek's Disease. They were transferred to pens with concrete floors and wood shavings for litter. Feed in mash form and water were allowed ad libitum. The antibiotic-free diet contained 20% CP and 2,685 kcal of ME/kg and was the same formulation as that fed to the parental lines. At 8 wk, chicks were weighed to the nearest gram and sex determined.

Data Analysis

Body weights were analyzed by ANOVA (SAS Institute, 2013) with log transformation because means and variances were correlated. The statistical model was:

$$y_{ijk} = \mu + G_i + S_j + (GS)_{ij} + e_{ijk}$$

where i = 2…17 generations, j = 1, 2 sexes, and k = 1, 2…n individuals per generation and sex. Because we expected significant generation effects because of differences in ages of breeders, we decided a priori to calculate correlations between mean body weight and generation (2 to 17) for each sex.

Coefficients of variation and percent sexual dimorphism (female/male) were calculated for generations 2 to 17. Values for the parental population and F_1 were not included, because they were the base and the cross of those that formed the AIL (i.e. recombinants would be in the F_2 and subsequent generations).

Comparisons for the parental with the F_1 involve the estimates of heterosis, which was using means. The formula and data were then same as those used by Williams et al. (2002) who did a more extensive analysis for a multitude of traits and made comparison computational methods.

$$\text{Heterosis} : (F_1 - [(P_1 + P_2)/2]/(P_1 + P_2)/2 \times 100$$

where F_1 represents the line cross offspring and P_1 and P_2 are the progeny from each of the two parental populations. Coefficients of variation were calculated for each generation.

RESULTS AND DISCUSSION

Phenotypic comparisons for an AIL may be viewed in the Mendelian context of 3 phases − the parental lines, the F_1 crosses, and subsequent recombinant generations. In this experiment, the parental lines were the S_{41} generation of the Virginia high and low lines that had undergone selection for the single trait 8-wk body weight. Means and standard deviations were 1,827 ± 120, 1,433 ± 101, 234 ± 52, and 168 ± 38 g for HWS and LWS males and females, respectively. Values for the F_1 crosses were 774 ± 104 and 627 ± 101 g for HWS x LWS males and females, respectively. For the LWS x HWS matings, respective values were 917 ± 101 and 716 ± 87 g. As expected, coefficients of variation (Figure 1) were lower for the F_1 than for the recombinant generations. Heterosis for 8-week body weight from the respective matings was -25, -22, -11, and -11%. The negative heterosis was consistent with previous crosses between these lines at this age (Williams et al., 2002), and its sign does not reflect "good" or "bad." Also, long-term selection may have purged genes associated with either high or low body weight in their respective lines (Haldane, 1954). In addition, heterosis at a chronological age may differ from that at a physiological stage (Liu et al., 1993) and is population specific (Fairfull, 1990).

In developing an AIL, a goal is to design and maintain a reproductive population of adequate size for phenotypic and genetic stability across generations. Although number of matings to produce specific generations ranged across generation as we tried different mating schemes after the F_4, we decided that single pair matings were necessary to minimize inbreeding. This seemed appropriate as the chosen trait had a moderate

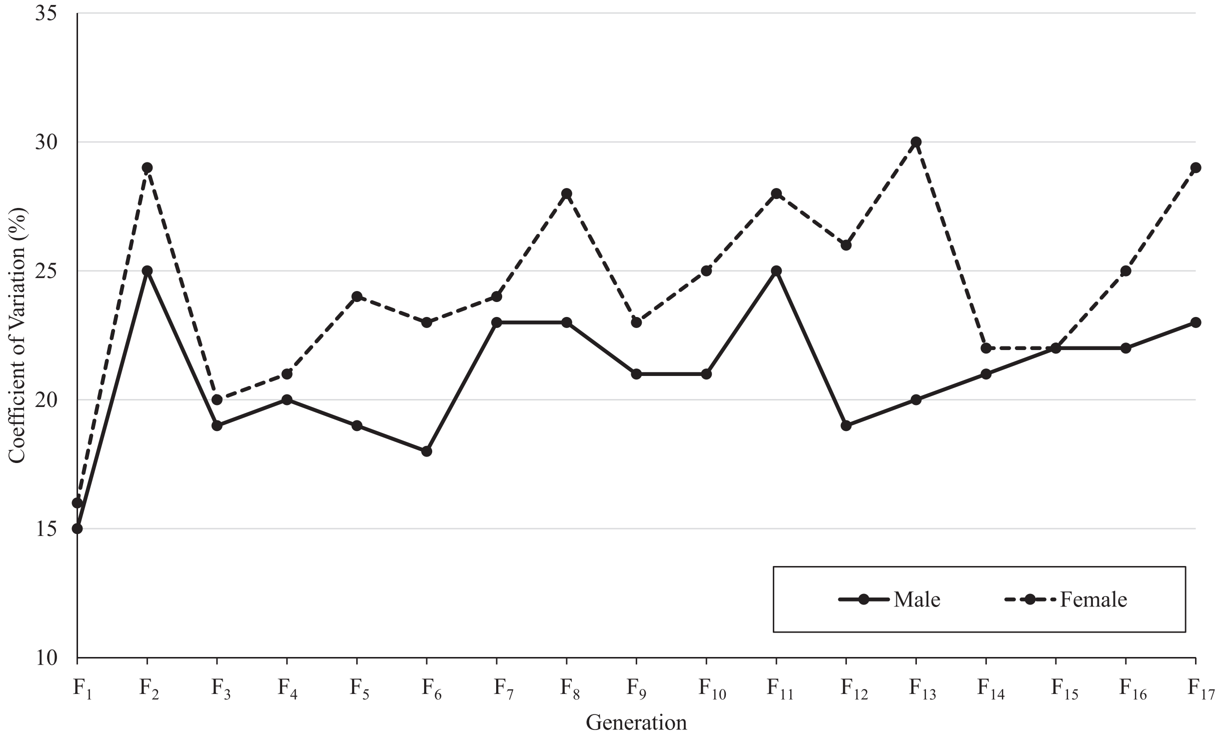

Figure 1. Coefficients of variation (%) for males and females for recombinant generations F_1 to F_{17}.

heritability. This was a good decision because we know that in our lines body weight is influenced by many genes, each with small effects (Jacobsson et al., 2005; Lillie et al., 2018).

Although there were fluctuations across recombinant generations, the pattern for body weight was the same for males and females (Figure 2) with no significant sex by generation interactions ($p > 0.05$). Correlations between body weight and generation 2 to 17 were -0.06 for males and -0.07 for females. Also, sexual dimorphism ranged from 73 to 80% across generations with the correlation between them of 0.02. This shows that there was no genetic trend by generation and that we have established an AIL that serves its purpose as a resource to study the segregation of alleles underlying the dramatic differences in body weight between the HWS and LWS

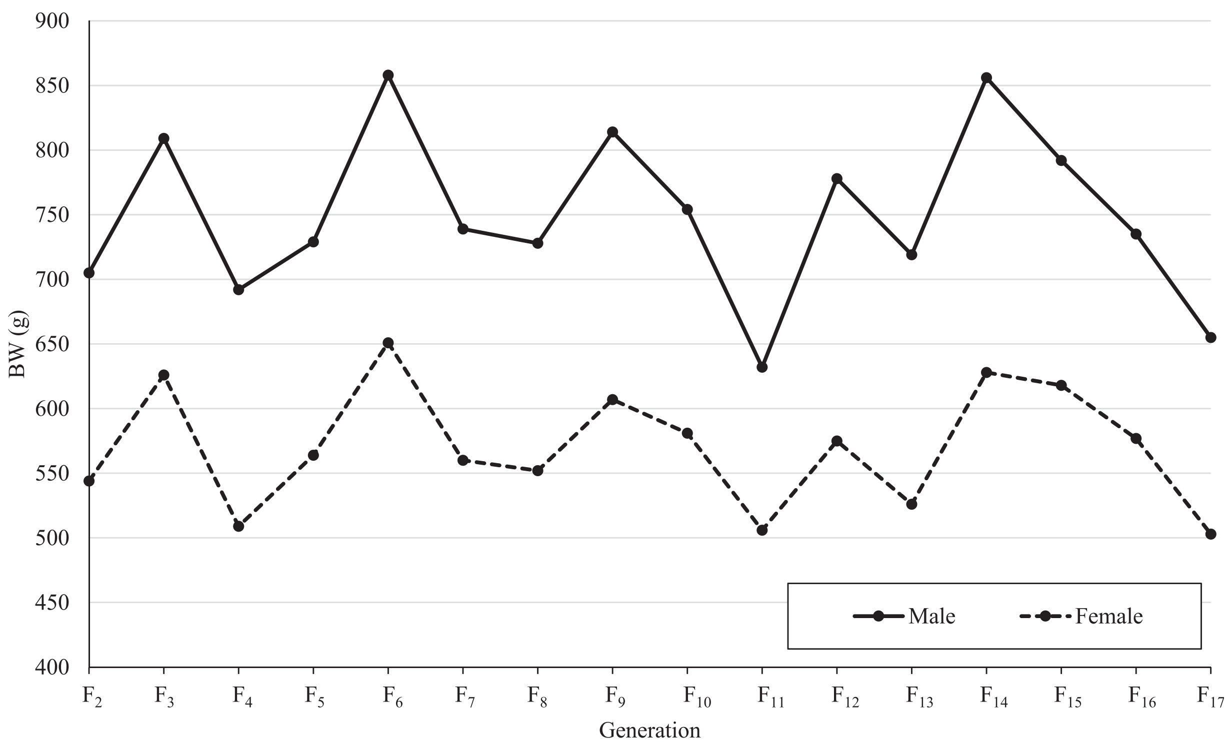

Figure 2. Mean 8-wk body weights (g) for males and females for recombinant generations F_2 to F_{17}.

lines. The fluctuations in body weight among generations are not surprising. They can be precipitated by factors such as environmental effects or age of breeder.

A literature on development of randombred populations of chickens was summarized by Hess (1962). These lines were designed to serve as a base and control for selection experiments. Because we were interested in various uses of our AIL per se we deviated from where the population would serve as a basis for a selection experiment per se. An example being the Virginia high and low antibody lines, which were derived from the Cornell Randombred population (e.g., Nolin et al., 2023). Thus, in our AIL, there were some "bottlenecks" due to fewer matings in some generations. An estimate was made for loss of heterozygosity (1/8 M) + (1/8 F), where M is the number of sires and F is the number of dams in each generation (Lush, 1949). By generation 17, the population may have been ~25% inbred.

Although intercross populations for genomic analyses have become common in the poultry literature, there is a dearth of information on phenotypic aspects of AIL. Moreover, there appears to be a general lack of studies where the parental lines were traced back, pedigree-wise, to a common founder. Juvenile body weight in chickens is a trait with moderate heritability influenced by many genes with small effects, thus reflecting the interface between genomic studies with phenotypic stability over the long term where sophistication in techniques for the former have occurred over time. Namely, the analytical techniques that are developed have and will continue to allow for further insights into the biological phenomena of AILs.

DISCLOSURES

The authors have no conflict of interest.

REFERENCES

Brandt, M., M. Ahsan, C. F. Honaker, P. B. Siegel, and Ö. Carlborg. 2017. Imputation-based fine-mapping suggests that most QTL in an outbred advanced intercross body weight line are due to multiple. linked loci. G3 7:119–128.

Carlborg, Ö., L. Jacobsson, P. Ahgren, P. B. Siegel, and L. Andersson. 2006. Epistasis and the release of genetic variation during long-term selection. Nature-Genet 38:418–420.

Darvasi, A., and M. Soller. 1995. Advanced intercross lines, an experimental population for fine genetic mapping. Genetics 141:1199–1207.

Fairfull, R. W. 1990. Heterosis. Pages 913−933 in Poultry Breeding and Genetics. R. O. Crawford, ed. Elsevier, Amsterdam, the Netherlands.

Gonzalez, N. M., and A. A. Palmer. 2014. Fine-mapping QTLs in advanced intercross lines and other populations. Mammal. Genome 25:271–292.

Haldane, J. B. S. 1954. The measurement of natural selection. Pages 480−487 in Proc. 9th Internat. Congress Genetics. London, England.

Harrison, S. J., P. B. Siegel, C. F. Honaker, and R. L. Lewis. 2023. Population dynamics of a long-term selection experiment in White Plymouth Rock chickens selected for low or high body weight. Poult. Sci. 102:102575.

Hasenstein, J. R., and S. J. Lamont. 2007. Chicken gallinacin gene cluster associated with *Salmonella* response in advanced intercross line. Avian Dis 51:561–567.

Heifetz, E. M., J. E. Fulton, N. P. O'Sullivan, J. A. Arthur, H. Cheng, J. Wang, M. Soller, and J. C. M. Dekkers. 2009. Mapping QTL affecting resistance to Marek's disease in an F6 advanced intercross population of commercial layer chickens. BMC Genomics 10:20.

Hess, C. W. 1962. Randombred populations of the Southern Regional Poultry Breeding Project. W. Poult. Sci. J. 18:147–152.

Jacobsson, L., H.-B. Park, P. Wahlberg, R. Fredriksson, M. Perez-Enciso, P. B. Siegel, and L. Andersson. 2005. Many QTLs with minor additive effects are associated with a large difference in growth between two selection lines in chickens. Genet. Res. 86:115–125.

Jennen, D. G., A. L. J. Vereijken, H. Bovenhuis, R. M. P. A. Crooijmans, J. J. van der Poel, and M. A. M. Groenen. 2005. Confirmation of quantitative trait loci affecting fatness in chickens. Genet. Sel. Evol. 37:215.

Lillie, M., Y. Sheng, C. F. Honaker, L. Andersson, P. B. Siegel, and Ö. Carlborg. 2018. Genomic signatures of 60 years of bidirectional selection for 8-week body weight in chickens. Poult. Sci. 97:781–790.

Liu, G., E. A. Dunnington, and P. B. Siegel. 1993. Maternal effects and heterosis for growth in reciprocal cross populations of chickens. J. Anim. Breed. Genet. 110:423–428.

Lush, J. 1949. Animal Breeding Plans. Iowa State College Press, Ames, IA.

Nolin, S. J., R. L. Taylor Jr., F. W. Edens, P. B. Siegel, and C. M. Ashwell. 2023. Combining supervised machine learning with statistics reveals differential gene expression patterns related to energy metabolism in the jejuna of chickens divergently selected for antibody response to sheep red blood cells. Poult. Sci. 102:102751.

Prashar, A., P. M. Hocking, J. T. Erichsen, Q. Fan, S. M. Saw, and J. A. Guggenheim. 2009. Common determinants of body size and eye size in chickens from an advanced intercross line. Exper. Eye Res. 89:42–48.

Pettersson, M., F. Besnier, P. B. Siegel, and Ö. Carlborg. 2011. Replication and explorations of high-order epistasis using a large advanced intercross line pedigree. PLoS Genetics 7:e1002180.

Redmond, S. B., P. Chuammitri, C. B. Andreasen, D. Palić, and S. J. Lamont. 2011. Genetic control of chicken heterophil function in advanced intercross lines: associations with novel and known *Salmonella* resistance loci and a likely mechanism for cell death in extracellular trap production. Immunogenet 63:449–458.

Rönneburg, T., Y. Zan, C. F. Honaker, P. B. Siegel, and Ö. Carlborg. 2022. Low-coverage sequencing in a deep intercross of the Virginia body weight lines provide insight to the polygenic genetic architecture of growth: novel loci revealed by increased power and improved genome-coverage. Poult. Sci. 102:102203.

SAS Institute Inc. 2013. SAS® 9.4. SAS Institute Inc., Cary, NC.

Sheng, Z., M. Pettersson, C. Honaker, P. Siegel, and Ö. Carlborg. 2015. Standing genetic variation as a major contributor to adaptation in the Virginia chicken lines selection experiment. Genome Biol 16:219.

Siegel, P. B. 1962. Selection for body weight at eight weeks of age: 1. Short term response and heritabilities. Poult. Sci. 41:954–962.

Van Goor, A., K. J. Bolek, C. M. Ashwell, M. E. Persia, M. F. Rothschild, C. J. Schmidt, and S. J. Lamont. 2015. Identification of quantitative trait loci for body temperature, body weight, breast yield, and digestibility in an advanced intercross line of chickens under heat stress. Genet. Sel. Evol. 47:96.

Wahlberg, P., Ö. Carlborg, M. Foglio, X. Tordoir, A.-C. Svyänen, M. Lathrop, I. G. Gut, P. B. Siegel, and L. Andersson. 2009. Genetic analysis of an F_2 intercross between two chicken lines divergently selected for body-weight. BMC Genomics 10:248–260.

Wang, Y., L. Bu, X. Cao, H. QU, C. Zhang, J. Ren, Z. Huang, Y. Zhao, C. Luo, X. Hu, D. Shu, and N. Li. 2020. Genetic dissection of growth traits in a unique chicken advanced intercross line. Front. Genet. 11:894.

Williams, S. M., S. E. Price, and P. B. Siegel. 2002. Heterosis of growth and reproductive traits in fowl. Poult. Sci. 81:1109–1112.

Zan, Y., Z. Sheng, L. Rönnegård, C. F. Honaker, P. B. Siegel, and Ö. Carlborg. 2017. Artificial selection response due to polygenic adaptation from a multi-locus, multi-allelic genetic architecture. Mol. Biol. Evol. 34:2678–2689.