Is The 'Double-Yolked' White Leghorn Line A 'Booroola' Hen ?

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Introduction

The number of eggs is a good indicator of ovulation rate in the chicken. Selection for egg production has led to a great increase in egg number, associated with a daily ovulation rhythm during most of the production year of commercial hens. Double ovulations are usually very rare and take place mainly in the first month of the laying period.
An experimental line of White Leghorn hens was selected for an increased number of double-yolked eggs (DY) by H. Abplanalp between 1963 and 1979 ((Lowry *et al*., 1979). Some eggs could even carry 3 yolks. Heritability of the DY trait was estimated to be 0.30 (Lowry et Abplanalp, 1967). Physiological studies showed that the double ovulation was mainly due to the simultaneous development of two follicules on the ovary, and that a single preovulatory peak of LH took place (Sharp *et al*., 1976; Lowry *et al*., 1979).
A subset of this line, thereafter called WLDJ, was imported to France by INRA in 1983 and maintained under a mild selection pressure for the DY trait, with some crossbreeding with other White Leghorn hens to avoid excessive inbreeding. Since it is not possible to hatch chicks from double-yolked eggs, WLDJ hens reproduce late when they lay some single-yolk eggs. The sex-linked dwarf gene was introduced in the line by H. Abplanalp, since dwarf sisters from double-yolked hens had much less abnormal eggs and reproduced more easily.

The analysis of the genetic determinism of multiple ovulations in mammals, particularly the sheep, has made important progress in recent years, with the identification of several mutations in genes controlling ovulation, such as *BMP15*, *BMPR1B* or *GDF9* (Fabre *et al*., 2006). Thus, the purpose of this study was to investigate the possibility that the phenomenon of multiple ovulations in the WLDJ was controlled by a major gene, and that homology could allow us to propose *BMP15*, *BMPR1B* or *GDF9* as candidate genes. To answer these questions, a few large families have been produced within the WLDJ line as well as in crossbreeding with unrelated lines, and a SNP of the *BMP15* gene has been genotyped within two large crossbred families.

Material and methods

Animals. The WLDJ line is maintained with 9 families of one sire and 5 dams each. It is segregating for the sex-linked dwarf gene (about 20% of the hens are dwarf) but this study will focus on the performance of normal hens only. Family size was increased in the year 2008 in order to study more precisely the variability of the DY trait within the line. Two sires

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were then chosen to produce crossbred families: they exhibited a large variability of the DY trait in their purebred daughters, they were not carrying the sex-linked dwarf gene and they were heterozygous for the *BMP15* marker. Sire #9644 was mated to 6 hens from an unrelated White Leghorn experimental line (WG) to produce a first set of crossbred hens (F1W) and it was mated to 10 hens from a commercial brown-egg layer line to produce a second set of crossbred hens (F1B). Sire #9722 was mated to another set of 10 hens from the same commercial brown-egg layer line, to increase the size of the F1B design. The unrelated WG line as well as the commercial layer line were not previously selected for multiple ovulations and exhibited an average rate of double-yolked eggs below 5%.

Performance recording. All hens were housed in individual cages at the age of 17 weeks. Lighting rhythm was set to 14 hours light/10 hours darkness. Hens were fed ad libitum with a diet containing 2680 Kcal energy, 17.5% raw proteins, and 3.5% calcium. The production period was set to 5 months for the pure line and F1W family, and to 7 months for the F1B families. Egg production was recorded daily with a portable device. The procedure considers several types of eggs, including normal, double-yolked, broken, double-yolked and broken, soft-shelled. Total egg number was calculated on the whole production period. Laying intensity was calculated for each hen as the ratio of total egg number on the total number of production days following the day of first egg. In order to correct for differences in recording period and in sexual maturity, the percentages of double-yolked (DY%) and soft-shelled eggs (SS%) were calculated for each hen. The broken double-yolked eggs were taken into account for DY%. Adult body weight was recorded at the end of the production period. Egg weight was recorded at 36 weeks of age for the pure line and F1W, and at a mean age of 34 weeks for F1B families. In addition, double-yolked eggs were weighed separately for F1B families.

***BMP15* polymorphism**. A preliminary study of the coding sequence of *BMP15* was conducted with 6 hens of the WLDJ line and revealed the presence of two SNPs (Vieaud *et al*., 2009). A pyrosequencing test was then designed to genotype a G→A SNP identified in exon 1. This SNP does not modify the protein sequence. It has been used here as a marker of the *BMP15* locus for an association study in the F1B design.

Statistical analysis. The normality of distributions was tested with the Univariate procedure of SAS™ . The effect of the *BMP15* genotype on egg production traits was tested with the GLM procedure of SAS™ using the following linear model: $Y = \mu + s_i + d_{ij} + b_k + e_{ijkl}$ where s, d, b are the fixed effects of sire, dam within sire and *BMP15* genotype respectively.

Results and discussion

Purebred performance. The DY% trait showed a very high coefficient of variance, close to 40%, and the total distribution tended to deviate from normality. It did not exhibit the usual asymmetric shape of a percentage trait, but rather a bimodal shape (figure 1). The largest sire families (19 to 25 hens) exhibited a similar level of within-sire variance. The percentage of soft shelled eggs was as high as the percentage of double-yolked eggs (table 1).

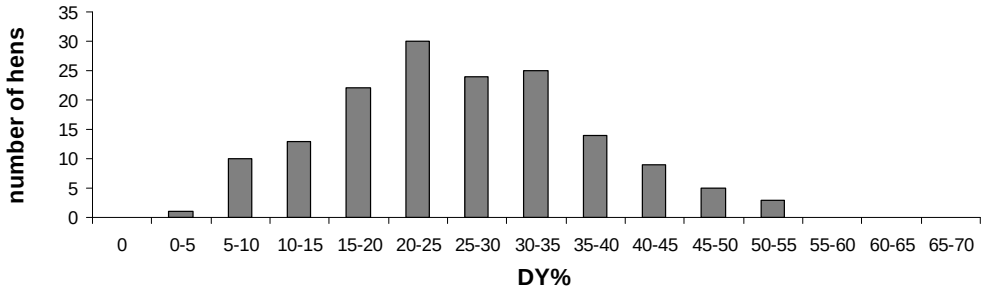

Figure 1: Distribution of the percentage of double-yolked eggs in the WLDJ line.

Table 1: Egg production traits of purebred WLDJ hens and crossbred hens.

Genetic group (size)	Age at first egg (days)	Laying rate	% double-yolked eggs (mini-maxi)	% soft-shelled eggs (mini-maxi)	Egg weight (g)	DY egg weight (g)	Body weight (g)
Purebred (n=113)	132.9 ± 7.2	81.7 ± 15.4	25.8 ± 11.3 (2.94 - 51.7)	28 ± 14 (3.7 - 65.4)	53.3 ± 5.6	-	1917 ± 214
F1W (n=43)	136.5 ± 4.9	93.7 ± 7.0	27.4 ± 9.5 (6.5 – 48.5)	23.3 ± 12.1 (3.7 – 51.3)	57.9 ± 4.7	-	2169 ± 182
F1B (n=139)	128 ± 5	93.7 ± 5.77	11.2 ± 7.8 (0 – 39.7)	8.6 ± 6.6 (0 – 38.2)	57.9 ± 3.7	78.7 ± 7.1 (n=73)	2225 ± 284

Crossbred performance. The value for the DY% trait of the F1W family was similar to that of the pure line, but it was much lower in the F1B families, which may be due to the longer recording period, considering that the rate of multiple ovulations decreases with age. Sire #9644 which produced the F1W family had still a higher value for DY% in the FIB design (13.9%) as compared to sire #9722 (8.6%). The percentage of soft-shelled eggs was also reduced as compared to the WLDJ line. Yet, both values were much higher than the usual performance described for the parental line of commercial brown-egg layers. Total egg number of F1B was higher than egg number of the WLDJ line, as a result of earlier sexual maturity and higher laying rate (+10%). Egg weight and body weight were also increased by 3.5% and 11% respectively.

The distribution of the DY% trait in F1W was bimodal as in the pure line, but was not so in F1B (figure 2). Yet, this trait does not take into account soft-shelled eggs, which often correspond to a double ovulation disconnected from the shell synthesis. Thus, the phenotype of multiple ovulations should also include 'twin' soft-shelled eggs. Since these were not systematically recorded, an approximate rate of multiple ovulations may be obtained by adding the percentages of double-yolked (DY%) and soft-shelled (SS%) eggs. The distribution of the cumulated percentage exhibited a bimodal shape for sire #9644 (figure 2).

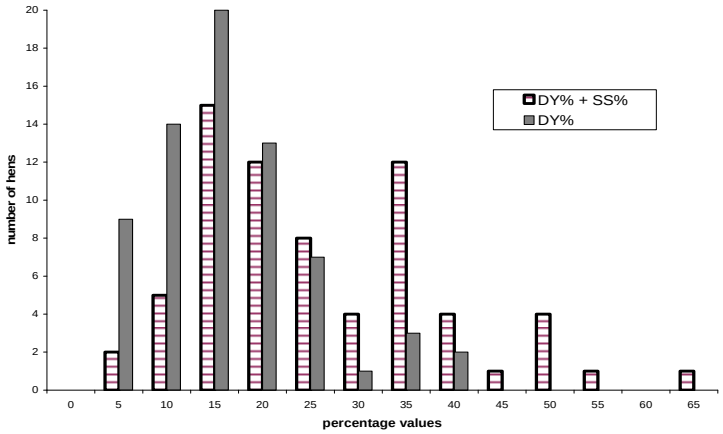

Figure 2: Distribution of the percentage of double-yolked eggs (solid bar) and the percentage of multiple ovulations, i.e. double-yolked + soft-shelled, (striped bar) in one crossbred family of the F1B design (69 hens from sire 9644).

***BMP15* polymorphism.** Alleles at the SNP marker were evenly distributed with 67 hens heterozygous A/G and 69 homozygous G/G; genotype was missing for 3 hens. The analysis of variance showed a significant effect of the sire ($p<0.01$) and of the dam ($p<0.05$) on the DY% trait but there was no effect of the *BMP15* marker. The R^2 of the model was 34% for the DY% trait. It was 25% for the SS% trait, where only the dam had a significant effect ($p<0.05$). The *BMP15* marker had no significant effect on any other egg production trait and can be excluded from further analysis of the mechanism of multiple ovulations in this line.

Conclusion

The WLDJ line is an interesting model for a comparative study of the genetic determinism of multiple ovulations between birds and mammals. The current results do not exclude the hypothesis of a major gene. The crossbred families produced by sire #9644 will be used to investigate association with other candidate genes and, if possible, for a whole genome scan.

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References

Fabre, S., Pierre, A., Mulsant, P. *et al.* (2006). *Reprod. Biol. Endocrinol*., 4:20.

Lowry, D.C. and Abplanalp, H. (1967). *Genetics*, 56:573-574.

Lowry, D.C., Dobbs, J.C., and Abplanalp, H. (1979). *Poult. Sci*., 58:498-501.

Sharp, P. J., Beuving, G., and Van Middelkoop, J.H. (1976). In *Proc. 5th European Poultry Conference*, 11:1259-1264.

Vieaud, A., Fabre, S., Gourichon, D. *et al.* (2009). In *8èmes J. Rech. Avicole en France*, 159.