Triploidy in broiler chickens: a brief review and case description

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Summary

Triploidy is a rare chromosomal abnormality observed in many species. According to literature, it may cause embryonic death, malformations, retarded growth and sterility in chickens. This study describes the identification of triploid broilers using allele intensity information from genotyping arrays, population incidence, and impact of triploid genotype on growth performance and patterns of inheritance within families in two broiler populations. Triploid genotypes presented high SNP call rates (~97%) which means that they can pass undetected by normal quality control steps. Furthermore, allele intensity clusters can be used for their identification. Triploid birds were, on average 13% and 15% lighter for populations A and B, respectively. Pedigree information suggests that there may be a pattern of inheritance for triploidy given that some grandparent families were more likely to produce triploids than others. Moreover, younger hens also showed higher incidence of triploid offspring. Even though it seems to be a rare anomaly in broilers, it should be better understood and monitored so that actions to prevent factors causing triploidy are taken in broiler breeding programs.

Keywords: broiler, triploid, chromosomal abnormalities

Introduction

Genomic technologies offers us the capability of screening populations for chromosome abnormalities and mutations to ensure these aberrations do not interfere with genetic improvement in a broiler breeder setting. Many types of chromosomal abnormalities have been described for *Gallus domesticus* in the literature such as aneuploidy, polyploidy, translocation, inversions and deletions (Bloom, 1972). The incidence of triploidy in both embryos and adult layer chickens has been reported by de Boer (1984), where the authors showed that triploidy could be linked to intersexuality in adults and was seen more frequently in embryos from eggs laid by very young hens (4.4%). Spontaneous masculinisation of hens was observed in complete triploid chickens with a ZZW sex chromosome constitution, what will likely prevent normal formation of oocytes and, as consequence, make them sterile (Frankenhuis, 1990). Moreover, the ZWW constitution has been described as lethal causing early embryonic death or malformed chick that are not expected to survive (Thorne, 1991). In comparison, the same authors showed that more than 50% of embryos ZZW and ZZZ were normally developed. The genetic basis for what can cause the formation of triploid embryos is not well understood, Thorne (1997) pointed out that a multifactorial mode of inheritance cannot be excluded. According to Fechheimer (1981), most triploids probably originate from suppression of the formation of the second polar body (resulting in ZZZ and ZWW) and

smaller proportions are due to suppression of the formation of the first polar body (resulting in a ZZW) or to fertilization off eggs with two sperm cells resulting in ZZZ and ZZW. The occurrence of such abnormality seems to have some hereditary influence given the rapid response to selection for triploidy, as shown in Thorne (1987). CSIRO created a synthetic line selected for triploidy that produced up to 20% triploid embryos (approximately 50 times greater than "regular" lines). They observed that females from the synthetic line ovulated a high incidence of diploid ova and this trait is genetically determined (Thorne, 1980). Thorne (1997) suggested that meiotic mutations at one or two loci are the most plausible genetic basis.

With the increasing availability of genomic information in individual birds within Cobb-Vantress breeding program, we were able to identify this rare condition in some live chickens. The objective of this study was to assess the incidence of triploid animals in broiler populations, evaluate the impact of such chromosomal abnormality on growth, and finally, if there is a hereditary pattern within broiler populations.

Material and methods

Blood samples were collected from two broiler populations at Cobb-Vantress. For each individual, 10ul of blood was collected into a blood cell stabilisation solution. DNA was extracted for each sample at Neogen Geneseek (Lincoln, Nebraska) and subsequently genotyped using Cobb's proprietary 60K Illumina SNP array. Genotype call rates were calculated for all samples. Triploid birds were identified by their lower SNP call rates. Genotype cluster files from suspected triploid samples were then assessed visually to confirm the presence of the extra cluster cloud expected in a triploid sample (Figure 1).

For all triploid samples identified and confirmed, extended pedigrees for up to three generations and body weight phenotype were used for further evaluations on the recurrence of triploidy within families and its impact on growth performance.

Results and Discussion

Triploid identification

SNP call rates on DNA samples from Cobb are generally higher than 99.91% due to the high quality and consistency of sample collection. Individual data files, and cluster files of samples falling below a call rate of 98% were manually checked to identify any specific data problems. Some of these individual samples revealed a cluster pattern consistent with triploidy (Figure 1a). In order to rule out sample contamination in these events, sample cluster files were also compared with contaminated samples (Figure 1b) and normal samples (Figure 1c).

On average, triploid samples had a SNP call rate of 97%. Such high call rates can make samples be classified as usable for most purposes and are also acceptable as a single sample result. However, the genotype calling will be incorrect in this case and, even though a sample can be clearly be categorized as triploid, diploid genotypes may be returned for it. Consequently, if such samples are not identified they could potentially introduce errors to any subsequent analysis.

Incidence of triploids in broiler populations

The incidence of triploids found in our data sets was extremely low (Table 1). Population A (Pop A) had more extensive information around triploids than population B (Pop B). Overall,

Pop. A showed, on average, a higher incidence of triploid birds (approximately 2 times higher) compared to B. For Pop. A, 1 in every 1667 birds presented this chromosomal abnormality while 1 in every 3505 birds showed the same anomaly for Pop. B. Those incidences are even lower than the ones reported by Thorne (1991) for layer strains (0.1-0.5%). There is no indication of a positive trend for this rare chromosomal disorder in broilers as frequencies in every contemporary group seem to fluctuate. Differences in incidence between these two populations could indicate a genotype effect.

It is important to highlight that the incidence of triploids for the whole population is unknown, since not all birds or unhatched embryos in those populations are genotyped. This study is only considering 3n birds that had normal development and were potential candidates to selection. Understanding the incidence and causes of triploidy can assist selection programs to cull families that are more likely to produce triploid progeny. As opposed to some species in which a triploid end-product is desirable given its ability to grow faster, having this chromosomal abnormality in chickens inside breeding populations is undesirable because it can affect embryonic mortality, growth and produce birds that are incapable of reproduction.

More than 80% of triploid birds were hatched from eggs produced by younger hens (<45 weeks of age). Therefore, hen's age seems to play a role in such anomaly, as already mentioned by de Boer (1984).

Table 1. Incidence and rate of birds showing triploid genotypes in two broiler populations (Pop A and Pop B).

CG	Pop A		Pop B	
	Incidence (%)	**Rate (1/n)**	**Incidence (%)**	**Rate (1/n)**
1	0.153	654	NA	NA
2	0.349	287	NA	NA
3	0.073	1368	NA	NA
4	0.063	1595	NA	NA
5	0.041	2452	NA	NA
6	0.071	1414	NA	NA
7	0.000	0	NA	NA
8	0.056	1784	NA	NA
9	0.019	5332	NA	NA
10	0.000	0	NA	NA
11	0.058	1739	NA	NA
12	0.070	1428	0.032	3084
13	0.065	1532	0.032	3096
14	0.064	1558	0.019	5316
15	0.168	596	0.150	668
16	0.043	2326	0.037	2707
17	0.106	940	0.016	6161

Impact of triploidy on growth performance, and recurrence within families

Body weight records show that triploid birds seem to have lower growth performance. In population A, birds that presented triploidy in genotyping QC were on average 14% (300g) lighter than birds in their contemporary group. Similar impact was seen for population B where triploids were 340g lighter when contrasted to their contemporary group means. Bloom (1972) noted not only growth retardation but also malformation can be associated with

chromosome aberrations, including triploidy. Another interesting point is that 90% of 3n birds were phenotypically classified as males. This is expected given that ZWW individuals don't survive and ZZW are seem as masculinised hens.

The occurrence of triploids within families indicates that some are more likely to produce triploid progeny than others when looking back at grandparent generation. One grandsire family in population A was responsible for 7% of triploids in the genotyped population. These results show the importance to evaluate the frequency of triploidy in dead embryos and malformed birds. A paper in zebra finch (Forstmeier, 2010) points to trisomy and triploidy as major sources of hatching failure.

Conclusions

Cluster files generated from allele intensity information from genotyping arrays can be used to identify triploid individuals in broiler population. Having tools to identify triploidy is important considering that most samples presenting this chromosomal abnormality will not fail normal quality control checks, such as call rate. The reported incidences for triploidy show that this is a rare anomaly but it should be monitored in order to prevent the selection of breeders that are more likely to produce triploid offspring. The background to producing 3n birds seems to be multifactorial, involving genetics, environment and hen's age.

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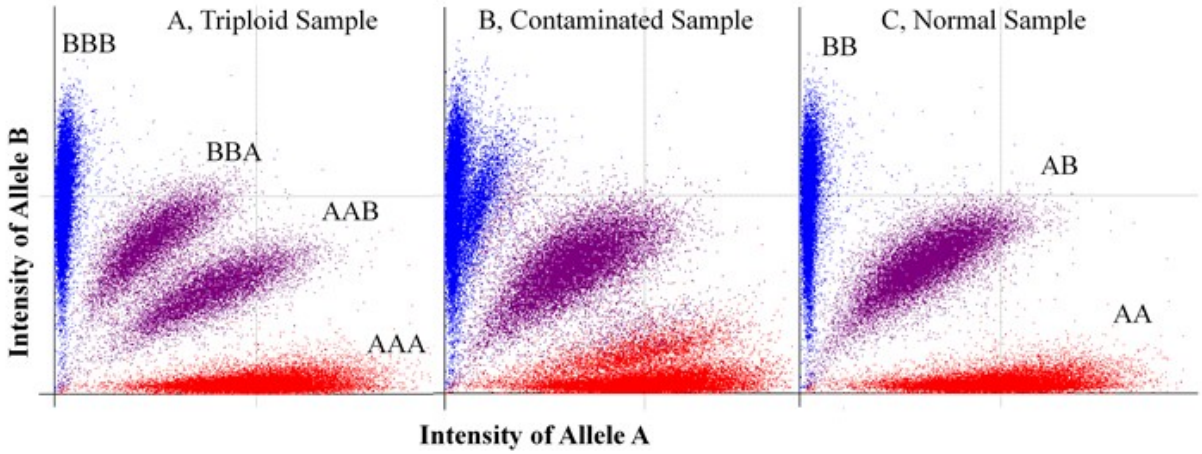

Figure 1. Cluster files of a (A) triploid chicken DNA sample, (B) contaminated DNA sample (i.e.: two chicken DNA samples), and a (C) normal single chicken DNA sample). Each dot represents the allele call for a single SNP. Blue dots are homozygous calls for the B allele. Red dots are homozygous SNP for the A allele. Purple dots are heterozygous SNP (AB).