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Efficient detection of interchromosomal rearrangements in Holstein bulls using large SNP datasets

J. Jourdain[1,2*], A. Pinton[3], H. Barasc[3], A. Barbat[2], C. Hozé[1,2], M. Philippe[4], D. Boichard[2] and A. Capitan[1,2]

[1]ALLICE, 75012 Paris, France; [2] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France; [3]GenPhySE, Université de Toulouse, INRAE, ENVT, 31320 Castanet-Tolosan, France; [4]EVOLUTION, 35538 Noyal-sur-Vilaine, France; [*]jeanlin.jourdain@inrae.fr

Abstract

Chromosomal structural rearrangements are the cause of reproductive problems. They can be transmitted between generations by progeny carrying a balanced translocation. Detection and elimination of carrier bulls and their carrier progeny is desirable to increase on-farm conception rates. In this paper, we propose to detect these chromosomal abnormalities based on a significant association disequilibrium between markers of different chromosomes in the progeny of a bull. This approach applied to 2445 Holstein artificial insemination (AI) sire families resulted in the detection of 10 candidate rearrangements (0.41% of bulls). Most of the suspected bulls had low conception rates (relative loss of 29-65% compared to breed average). While historically, translocation studies in cattle focused on targeted cases, our method can be used routinely to detect chromosomal rearrangements based on SNP genotypes available for genomic evaluation.

Introduction

Chromosomal abnormalities have been reported in many livestock species (*e.g.* Mallepaly *et al.*, 2017) with serious negative consequences for health and reproductive performances. In contrast to other species, in cattle, there are few systematic cytogenetic controls by banding, which probably leads to an underestimation of the phenomenon (De Lorenzi *et al.*, 2012). To date, only 20 reciprocal translocations and 42 Robertsonian fusions have been reported (Iannuzzi *et al.*, 2021). In this paper, a new routine method, using genomic information from halfsibs families, is proposed to detect structural rearrangements in AI sires.

Materials & Methods

Genomic and cytogenetic analyses. Phased and imputed Illumina Bovine SNP50 genotypes were obtained from the French genomic evaluation database as described in Mesbah-Uddin *et al.*, 2019. We considered a total of 2,445 Holstein paternal families comprising from 30 to 12,600 halfsibs, all genotyped in France. The average number of informative SNPs on the 29 autosomes was 14,561 ± 607 per bull. Genomic data were processed with R 3.6.2. Association correlations between SNPs were calculated using R package gaston 1.5.6. Significance thresholds under H0 were obtained empirically as follows: 100 bulls with more than 1,500 genotyped offspring and with good reproduction scores (*i.e.,* assumed to have normal karyotype) were selected. For each bull, 10 samples of n progeny were randomly drawn for 12 given values of n between 25 and 1000. For each halfsib family of n individuals, a two-dimensional genome scan was used to detect the maximum correlation between markers of two distinct chromosomes (Rmax). This Rmax was scored if 19 informative adjacent markers on each chromosome had R levels values greater than 0.75 Rmax. If this condition was not met, we considered the next highest correlation between markers of distinct chromosomes and so on. For a given n, 1,000 (=10*100) halfsib families were analysed. Thresholds corresponding to $p<0.001$ were obtained for any n between 25 and 1000 by fitting a regression curve to these data (n, Rmax). These thresholds, ranging from 1 to 0.24 for 30 to 1,000 halfsibs, enabled us to

detect bulls with putative chromosomal rearrangements. Correlation graphs displayed in the results were plotted with ggplot2 3.0.0. package of the R software. Finally, karyotypes of sire C and eight daughters of sire E were obtained from blood lymphocytes as described in Ducos *et al.*, 1998.

Reproduction data. Out of the 2,445 sires considered, the conception rate (CR, *i.e.,* the number of calving occurring between 264 and 294 days after AI divided by the number of AIs) and non-return rates (NRRx; defined as proportion of cows not re-inseminated x days after AI) were calculated for 2,252 bulls that totalized at least 200 AIs with conventional semen between 2010-01-01 and 2020-12-31 (35 million AIs considered).

Results and Discussion

In our dataset, ten bulls (0.41%) were suspected to carry a structural rearrangement with $p < 0.001$ (Table 1). Surprisingly, none of them was Robertsonian, contrarily to what would be expected in a species with only acrocentric autosomes. The estimated size of the translocated segments ranged from 2 to 25 Mb (mean = 10.3 ± 8.2 Mb) and 15 out of the 29 autosomes were affected with two occurrences for BTA2 and three for BTA8 and BTA12.

Table 1: Characteristics of the 10 putative rearrangements

Bulls ID	A	B	C	D	E	F	G	H	I	J
Affected chromosomes	2,13	10,12	3,8	5,28	4,8	24,29	2,12	6,8	11,16	12,23
Genotyped progeny	36	35	512	68	519	59	49	100	43	41
CR (rank among 2,252 bulls in %)	21.8 (99.7)	24.1 (99.6)	21.2 (99.8)	14.3 (100)	28.7 (99.2)	21.5 (99.8)	15.7 (99.9)	43.8 (29.0)	26.1 (99.6)	21.6 (99.7)

We have begun collecting blood samples from these sires or their offspring for karyotyping. At time of writing, we had results for 2 bulls, confirming our findings. For bull C, we observed a reciprocal translocation between BTA 3 and 8 in both analyses (Figure 1a). For bull E, delineation of the rearrangement boundaries was made difficult by a run of homozygosity on BTA8 (b, up) and the small size of the segments involved. We observed additional material only on BTA8 (dark band) in the karyotype of some of the daughters. We hypothesize that this rearrangement is a simple insertion of chromosomal material from BTA4 into BTA8.

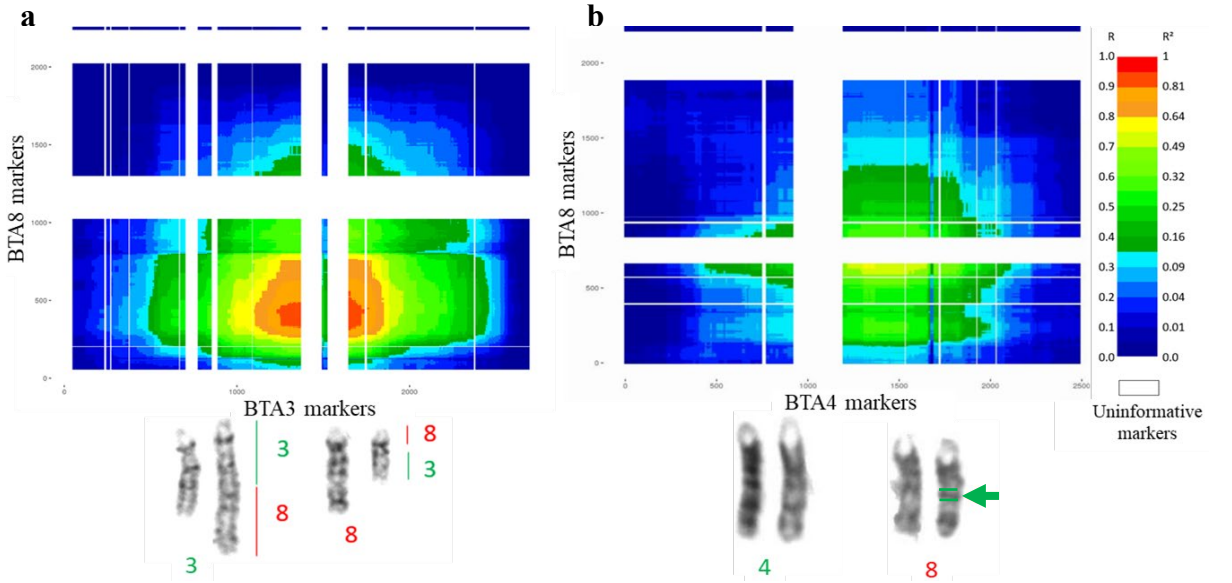

Figure 1: Linkage disequilibrium map of affected chromosomes (up) and details of karyotype (down) for bulls C (a) and E (b).

To precisely identify breakpoints and to search for motifs in their vicinity that could have favored chromosomal rearrangements, genomes of bulls A to J will be sequenced using PacBio CLR technology.

In a second step, we studied the reproductive performances of these ten bulls. Heterozygous carriers of structural chromosomal abnormalities, when they are fertile, can produce balanced (normal, rearranged) and unbalanced (with nullisomy and disomy) gametes. In most cases, unbalanced gametes cause early embryos or fetuses death (Raudsepp and Chowdhary, 2016). Nine bulls had a CR within the lowest percentile of their breed (Table 1) and represented 40% (9/2,252*100) of this worst percentile. Analyses of NRR over time supported early embryonic loss with distinct profiles (figure 2).

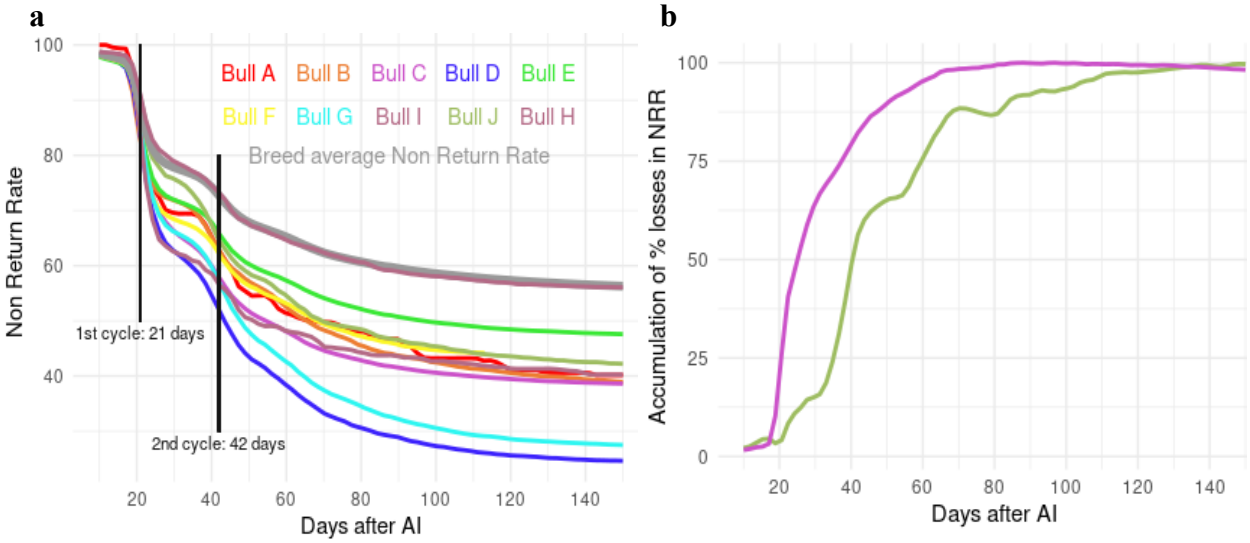

Figure 2: Non-Return Rate vs. breed average (a) and daily accumulation of the proportion of losses in NRR for bulls C and J (b).

For example, despite their low reproductive scores, bull E and D showed marked differences in the decrease of NNR as compared with the breed average, and thus of embryo and fetal losses (Figure 2a). Daily cumulative proportions of NRR loss also showed a diversity of patterns with bulls losing embryos rapidly (Bull C) versus bulls suspected of causing late fetal death (Bull J; Figure 2.b). This could be explained by the possible effects of rearrangements on semen production (Raudsepp and Chowdhary, 2016) and the diversity of consequences on early embryo or late fetal death (during organogenesis) depending on the genes carried and the size of the rearranged regions (Priya *et al.*, 2018). In our results, one bull (H) did not show evidence of reduced fertility. Further investigation should enable us to determine if this was a false positive or if this animal carries a rearrangement compatible with the survival of aneuploid conceptuses up to birth.

The high consequences on bull fertility of the rearrangements we have identified show that breeding companies should be immediately notified in case of high return rate (in worst 1% of the breed) and the suspect bulls should be at least karyotyped before continuing to sell their semen. Regardless of the genomic evaluation of sires, we advise companies to cull corresponding bulls to avoid a wide spread of abnormalities with long-term and costly consequences on the dairy sector. Non-carrier bulls can of course, be selected for the next generation.

The method we propose in this communication may underestimate the occurrence of chromosomal abnormalities. Indeed, chromosomal abnormalities can cause sterility by

azoospermia (Ghieh *et al.*, 2021). On the other hand, fertile bulls, because of their low fertility, might be prematurely culled because of their low NRR. They also have fewer calves and therefore fewer calves to be genomically tested by breeders, which decreases the accuracy and detection power of the method. We are working on complementary methods to detect suspect bulls based on genotypes of less than 30 daughters.
Despite this possible underestimation, our results are similar to studies in other species. Indeed, in human and swine populations, approximately 0.1 to 0.8% of individuals would carry a *de novo* chromosomal rearrangement (Barasc *et al.*, 2016; Tucker *et al.*, 1994). Furthermore, in humans, chromosomal abnormalities are estimated to cause half of embryo and fetus losses (Pylyp *et al.*, 2018).

Conclusions and perspectives

Chromosomal abnormalities have serious consequences for the dairy industry, especially with the high selection intensity of genomic breeding programs, narrowing down the number of bulls while allowing massive use of their semen before detecting problems with progeny tests. On the other hand, the large datasets generated in the framework of the genomic evaluation allow detection of rearrangements with high efficiency and higher resolution than the karyotyping banding method (5 to 10 Mb). The method proposed in this article can be used routinely to identify bulls with abnormal karyotypes and their balanced progeny to manage these defects in selection.

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