Ex Situ Conservation Of Holstein-Friesian Cattle - Comparing The Dutch, French And USA Germplasm Collections

C. Danchin-Burge[*], H. Blackburn** and S.J. Hiemstra***

Introduction

The establishment of gene banks using cryopreservation to secure the genetic diversity of farm breeds have been widely assessed (Blackburn 2004, FAO 2007). France, the Netherlands and the USA were among the first countries to organize national cryobanks (Blackburn 2004, 2009; Danchin-Burge *et al*., 2006; Woelders *et al*., 2006) and these banks are now 10 to 20 years old. All three countries have started Holstein-Friesian (HF) collections to conserve as much genetic diversity as possible for this globally important breed. In order better understand the diversity captured in these collections the: genetic variability of HF collections within and between countries was assessed, and genetic variability of germplasm collections were compared with active bulls in each country. The overall aim of the project was to determine the breed's security and to guide future collection activities.

Material and methods

Establishment of national HF germplasm collections was started at the beginning of the nineties for the Dutch cryobank and in 1999 for both the American and French Cryobank. For the Dutch collection, the majority of the bulls were on the Holland Genetics (now CRV) and Alta Genetics progeny testing program. The USA collection consists of bulls sampled from the sire evaluation programs of three major AI companies, in addition to donations of old semen by the industry and experimental populations from university scientists. In France, the main selection objective for bulls to enter the cryobank was to preserve the possibilities to evolve in the future by combining the preservation of genetic gains, plus selected and neutral genetic variability within the HF population (Verrier *et al*., 2003). The pedigrees of the HF bulls stored in all three collections (by January 2009) were provided by each country (Holstein Association USA for the American collection, CRV for the Dutch collection, and INRA-CTIG for the French collection). Their genetic variability was assessed by using the pedigree data to calculate measures of genetic diversity such as equivalent number of generations (EqG), probability of gene origin (effective number of founders, $f_{e;}$ effective number of ancestors, $f_{a;}$ main ancestors contribution; number of ancestors contributing the most for a cumulated expected contribution of 50% of the genes, N_{50}), inbreeding and kinship. The software PEDIG (Boichard, 2002, 2007) was used to calculate these parameters. To compare the genetic variability of the cryobank bulls with the active male population, we

[*] INRA, UMR 1313 Génétique Animale et Biologie Intégrative, 78350 Jouy-en-Josas, France
** National Animal Germplasm Program NCGRP-ARS-USDA 1111 S. Mason St. Ft. Collins, CO. USA
*** Centre for Genetic Resources, the Netherlands (CGN), Wageningen University and Research Centre, P.O. Box 65, 8200 AB Lelystad, The Netherlands

sampled a population in each country from the INTERBULL database with the following criteria. Each bull had to be born in the country where it was used; both parents of the bull were known; and birth years ranged from 2002 till 2006 (5 years, an equivalent of one generation interval).

Results and discussion

Table 1: Number of cryobank and active bulls and their pedigree completeness (equivalent number of generations, EqG), by country

Collection	No. of cryobank bulls (CBN)	EqG CBN	No. of active males (AM)	EqG AM
France (FRA)	144	9.4	3,286	10.1
The Netherlands (NLD)	3,755	9.3	2,257	9.6
USA (USA)	5,013	7.3	7,389	9.4

The number of bulls present in each collection and their pedigree size is described in table 1. No bull was stored in more than one national collection. The USA and Dutch collections are comparable by their size; however their composition is very different. The majority of the Dutch bulls were born in the nineties or the years 2000, while about 2/3 of the USA bulls were born in the eighties and the nineties, the last third being divided between the seventies and the years 2000. As for the French bulls, they were born in the nineties or 2000. The average birth year is: 1989, 1998, and 2000 for the USA, French and Dutch collections, respectively.

The pedigree completeness level was assessed for all the bulls by computing their traceable equivalent number of generations (EqG, table 1). The EqG is similar for the French and the Dutch collections, but the lower EqG of the American collection is explainable by the birth year distribution of the bulls: on average there were less ancestors known for bulls born in the sixties or the seventies than for bulls born in the nineties.

Table 2: Comparison of the effective number of founders (f_e), effective number of ancestors (f_a), contribution of the main ancestor (C1), and number of ancestors contributing the most for an expected contribution of 50% of the genes (N_{50}), between cryobank (CBN) bulls and the active male population (AM) by country

	Overall		FRA		NLD		USA	
	CBN	AM	CBN	AM	CBN	AM	CBN	AM
f_e	1,237	113	100	105	114	115	784	115
f_a	84	17	14	15	18	17	77	17
C1, %	4.8	14.1	14.6	14.6	13.5	13.1	5.3	14.2
N_{50}	43	6	5	6	7	7	53	6

When comparing all three collections (table 2) it is striking to see the similarity in genetic variability between the Dutch and French collection. For example, the number of ancestors contributing the most for a cumulated expected contribution of 50% of the genes (N_{50}) is equal to 5 and 7 for the French and Dutch cryobank, respectively, and these main ancestors are the same for both countries. The genetic variability represented by the American

collection is relatively high compared to France and the Netherlands. The N_{50} is equal to 5, 7 and 53 for the French, Dutch and American collections, respectively. These numbers are equal to 6, 7 and 6 respectively for the French, Dutch and US active male population. This result shows that most of the genetic variability of the HF breed seems well represented by the collections when comparing the male active population with the cryobank bulls.

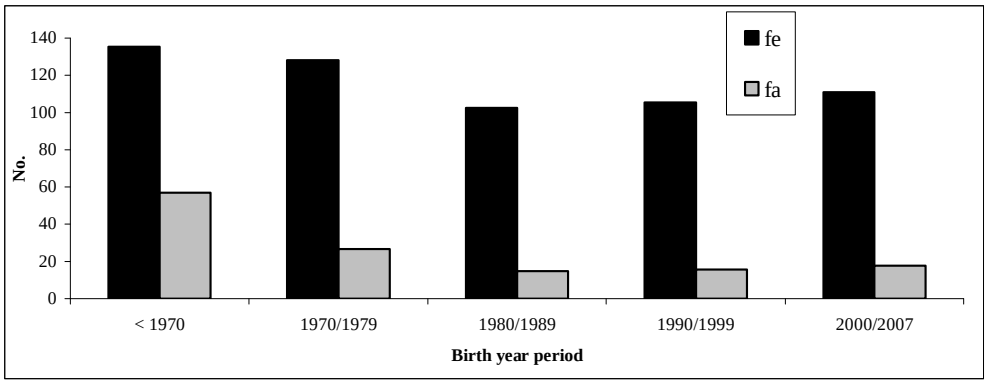

Figure 1: Evolution of the effective number of founders (f_e) and effective number of ancestors (f_a) by birth period of the cryobank bulls (all collections)

The f_e and f_a are decreasing respectively by 20% and 53% between the oldest bulls (born before 1970) and the bulls born in the seventies, and by 18% and 69% when compared to the youngest bulls. Since most old bulls belong to the USA collections, these animals most likely contribute to the higher genetic variability of the USA collection when compared to the live population.

Table 3: Average kinship ϕ (%) within and between each collection

Collection	FRA	NLD	USA	FRA*NLD	FRA*USA	NLD*USA
ϕ, %	6.4	5.0	4.8	5.0	4.9	4.2

Average kinship within collections (Table 3) showed the French collection has the most closely related bulls and the USA collection the least. Fifty bulls from the USA collections are donors from a randomly mated population developed by the University of Minnesota (Starkenburg et al., 1997) which contribute to the lower average kinship of the USA collection. The French collection is more highly related to the Dutch and USA collections: the French bulls are as related with the Dutch collection as the Dutch bulls among themselves. The Dutch and USA gene bank bulls are slightly less related, mostly due to the presence of old bulls in both collections which are quite disconnected.

The average kinship of the least related cryobank bulls in the French collection with the active population is over 4% (Table 4). It is equivalent to animals that have at least a great-grand-parent in common. The highest kinship values for all collections are for bulls that have on average a common grand-parent with all the active animals. However, some Dutch and American bulls are completely disconnected from the active male population. Apart from the

bulls from the University of Minnesota's "Control Line", some USA males are old bulls whose lineages are extinct today in the active pedigrees. Since the three collections are related, particularly with the more current bulls (data not shown), future collaboration might be useful to avoid collecting genetically similar bulls. However, such duplication also insures the maintenance of genetic diversity for such an important breed. It is recommended to carry out a careful investigation of the unrelated bulls to see if they can be used to help maintaining the HF breed's genetic variability.

Table 4: Average kinship ϕ (%) with lowest and highest (%) between each collection (CBN) and the active bulls (AM) of their country

Collection	ϕ between CBN and AM, %	Lowest value, %	Highest value, %
FRA	5.9 %	4.1%	7.2%
NLD	4.9%	0.0%	6.9%
USA	4.6%	0.0%	7.8%

Conclusion

The data suggest that the three national cryobanks have captured substantial amounts of genetic diversity for the HF breed when compared to the current active populations. A component of the USA, French and Dutch collections appear to be genetically similar. On the other hand, the USA collection represents an interesting reservoir of HF gene from the past; illustrating how gene banks can support the conservation of genetic diversity. In order to avoid duplication of efforts, it is suggested that cooperation between national cryobanks be increased. Further quantification of genetic diversity captured in the collections could be obtained via a molecular marker comparison with the in situ population.

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