



Holstein Friesian genetic introgression and its association with estimated breeding values and expected producing ability in Karan Fries cattle

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ABSTRACT

Introgression levels inform us about the admixture of genes within the genome of 2 populations due to crossing among them. The present investigation was carried out to determine the introgression levels of Holstein Friesian (HF) into crossbred Karan Fries (KF) cattle, developed under controlled breeding programme at National Dairy Research Institute (NDRI), Karnal, India. The association of HF introgression levels with estimated breeding values (EBVs) and expected producing ability (EPA) of KF cattle were also estimated. Eighteen reported microsatellite markers were used in the study and the HF introgression levels were assessed through STRUCTURE 2.3.4 program. Inheritance percentages of HF in the studied KF cattle showed varying degree of levels ranged between 50 and 75%. The results showed that the average HF introgression per cent in KF cattle was 52.30% ranging from 15.96 to 87.21%. The EBV and EPA showed significant association with increasing HF introgression levels. However, HF inheritance levels had nonsignificant effect on those traits. Introgression levels may be the future tool for explaining the productive performances of crossbreds than inheritance levels.

Key words: Crossbred, EBV, EPA, Inheritance, Introgression, Microsatellite

Genetic introgression is the movement of one species' genetic material into the gene pool of another and thereby producing a population with genetic novelty (Rheindt and Edwards 2011). An interbred hybrid with novel genome structure was developed as a new strain through the crossbreeding programme using Holstein Friesian (HF) semen as exotic germplasm on indigenous Tharparkar (TP) breed of cattle that was initiated at National Dairy Research Institute, Karnal in 1971. The half-breds (1/2 HF × 1/2 TP) born to the crossing were further inseminated with Holstein Friesian semen to obtain crossbreds with 75% exotic inheritance (three-quarter breds) (3/4 HF × 1/4 TP). In 1982, crossbreds with >50% exotic inheritance were merged together. The new synthetic cattle strain developed thereafter was nominated as "Karan Fries". The level of exotic inheritance in Karan Fries cattle, present at NDRI herd, is being maintained at varying levels between 50 and 75%. This study represents the foremost molecular survey of crossbreds produced from crossing between *Bos taurus* and *Bos indicus* in a designed crossbreeding programme in India for testing introgression of the exotic gene into indigenous breeds through artificial insemination. This study also tests for the association of introgression with

estimated breeding values (EBVs) and expected producing ability (EPA) of Karan Fries cattle.

MATERIALS AND METHODS

Sample animals: Blood was collected aseptically from 167 Karan Fries and 11 Tharparkar cattle from the institute herd. The studied Karan Fries cattle were of different filial groups which had completed their first lactation. In addition semen sample was collected from 11 Holstein Friesian bulls from Artificial Breeding Research Centre, NDRI.

Laboratory techniques: Genomic DNA from blood was extracted using standard phenol chloroform extraction method. DNA from semen of Holstein Friesian bulls was extracted as per Hossain *et al.* (1997). Eighteen unlinked bovine microsatellite loci (Table 1), selected from the BovMAP database, INRA, France were used to genotype all the samples (Qi *et al.* 2009). The allele size differences of the microsatellite loci at different loci among Karan Fries, Tharparkar and Holstein Friesian were examined from the microsatellite chromatogram through GENEMAPPER 3.0 program. STRUCTURE 2.3.4 program was used to detect the introgression level (Pritchard *et al.* 2000). The Holstein Friesian inheritance levels in studied 167 Karan Fries cattle population were traced back to pure HF breed from the history-cum-pedigree sheets maintained at Dairy Cattle Breeding Division of the Institute.

Statistical analysis: EBV and EPA data of 167 animals, based on their First lactation 305 days milk yield along

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Table 1. Eighteen microsatellite loci and their PCR conditions and allele size ranges in Karan Fries cattle population

S. No.	Locus Name	Chr*	Primer sequence (5'-3') Forward (above) and Reverse (below)	Forward primer label	A.T. (°C)	Allele size range (bp)
1	MGTG4B	4	GAGCAGCTTCTTTCTTTCTCATCTT GCTCTTGGGAAGCTTATTGTATAAAG	6-FAM	55	114-138
2	MGTG7	23	TTCATTGCAGCACTATTTACAATAG TAAGTTCCCTGTATCATTTTTGAA	TET	55	282-296
3	TGLA126	20	CTAATTTAGAATGAGAGAGGCTTCT TTGGTCTCTATTCTCTGAATATTCC	HEX	55	109-121
4	AGLA293	5	GAAACTCAACCCAAGACAACCTCAAG ATGACTTTATTCTCCACCTAGCAGA	6-FAM	55	220-242
5	CSSM066	14	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	HEX	58	96-106
6	BM2113	2	GCTGCCTTCTACCAAATACCC CTTCCTGAGAGAAGCAACACC	TET	58	125-145
7	BM1824	1	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACCTGCTTCCTTG	TET	58	182-194
8	ILSTS028	11	TCCAGATTTTGTACCAGACC GTCATGTCATACCTTTGAGC	6-FAM	55	129-159
9	TGLA122	21	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	HEX	55	137-165
10	TGLA53	16	GCTTTCAGAAATAGTTTGCATTCA ATCTTCACATGATATTACAGCAGA	TET	55	155-171
11	ILSTS008	14	GAATCATGGATTTTCTGGGG TAGCAGTGAGTGAGGTTGGC	6-FAM	58	173-183
12	ETH225	9	GATCACCTTGCCACTATTTCCCT ACATGACAGCCAGCTGCTACT	HEX	65	144-160
13	TGLA57	1	GCTTTTAAATCCTCAGCTTGCTG GCTTCCAAAACCTTTACAATATGTAT	6-FAM	55	77-99
14	ETH152	5	TACTCGTAGGGCAGGCTGCCTG GAGACCTCAGGGTTGGTGATCAG	TET	55	194-210
15	TGLA73	9	GAGAATCACCTAGAGAGAGGCA CTTCTCTTTAAATTCTATATGGT	HEX	55	119-143
16	ILSTS050	2	AAATCAGACACCCAGTTTCC GTTTTTCTACACGAGTTGGC	6-FAM	55	148-162
17	ILSTS013	9	CTTGATCCTTATAGAAGTGG ACACAAAATCAGATCAGTGG	HEX	55	111-125
18	SPS115	15	AAAGTGACACAACAGCTTCTCCAG AACGAGTGTCTAGTTTGGCTGTG	TET	65	242-260

* Cattle chromosome numbers are given; AT, annealing temperature for PCR amplification.

with their introgression level information were used for the association study. The data were adjusted through LSML Program taking account of age at first calving (AFC), season of calving and period of calving as non genetic factor along with exotic inheritance and exotic introgression as genetic factor. The regression of HF introgression levels on these 2 quantitative traits were assessed by applying the command PROC REG of SAS 9.3 software program using the following univariate regression model:

$$Y_{ij} = \alpha + \beta X_i + \epsilon_{ij}$$

where, Y_{ij} , observed EBVs and EPA value of each animal; α , mean EBVs and EPA value; β , regression of EBVs or EPA on assessed HF introgression levels; X_i , HF introgression/ inheritance level of each animal; ϵ_{ij} , random error associated with each record and assumed to be NID (0, σ^2_e).

RESULTS AND DISCUSSION

Holstein Friesian inheritance levels in Karan Fries cattle: The exotic inheritance level in the Karan Fries population varied from 50 to 75% (Table 3). The exotic inheritance varied due to crossing and backcrossing among 1/2 HF, 3/4 HF, 5/8 HF or, 7/8 HF inheritance level cattle. The pedigree information exerts that the Karan Fries cattle belong to F_1 to F_{11} generations.

Genetic introgression analysis: Specific allele size length of 16 microsatellite markers (except TGLA57 and TGLA 73) in Holstein Friesian, Tharparkar and Karan Fries cattle were different in 3 breeds (Table 2). From these allele lengths difference among the breeds, the admixture of Holstein Friesian in the Karan Fries cattle was estimated. The Holstein Friesian introgression levels in each Karan Fries cattle are depicted in Table 3. The average Holstein Friesian genetic introgression level in the studied Karan

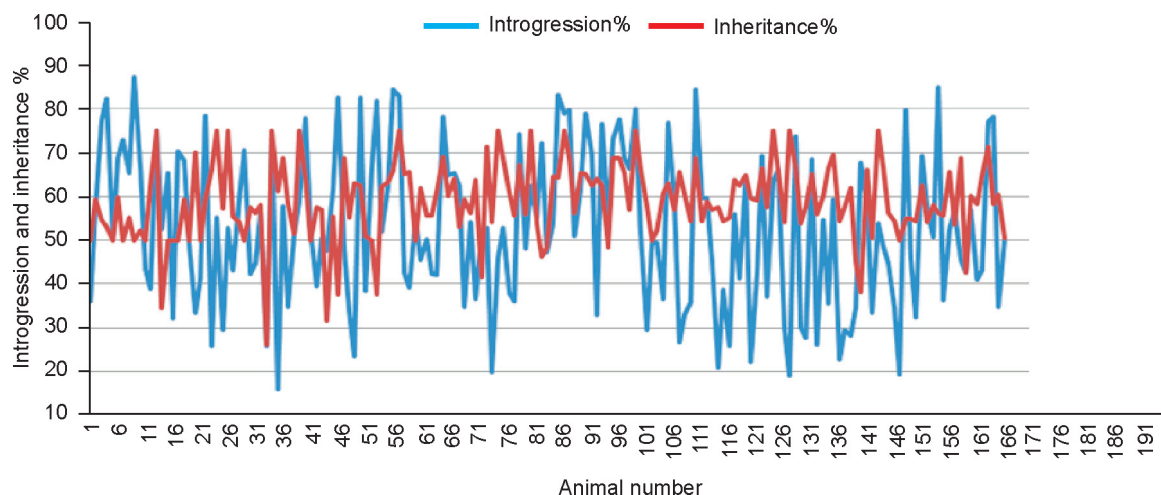


Fig.1. Variation between HF introgression and inheritance % levels in Karan fries cattle

Fries cattle population at NDRI was 52.30% ranging from 15.96 to 87.21%. The introgression levels were found highly variable in all the KF animals belonging to different filial generations. Even the animals having same level of HF inheritance, showed varying levels of HF introgression. The exotic introgression levels do not depend upon the exotic inheritance level in the Karan Fries cattle and showed independency between them. Even in the same filial generation the introgression levels was varying with the inheritance levels (Table 3). The high variation within the exotic inheritance and exotic introgression of Karan Fries cattle is depicted in Fig. 1.

Microsatellite analysis of genetic diversity provides 2 distinct levels of information (i) allele frequency differences among populations and (ii) relationships between alleles and groups of alleles on the basis of differences in allelic

Table 2. Allele size length differences in Holstein Friesian, Tharparkar and Karan Fries cattle

S. no.	Microsatellite locus	Holstein Friesian	Tharparkar	Karan Fries
1	MGTG4B	134–138	118–138	114–138
2	MGTG7	308–310	282–310	282–296
3	TGLA126	109–119	115–121	109–121
4	AGLA293	230–240	228–240	220–242
5	CSSM066	98–104	100–104	96–106
6	BM2113	127–139	131–143	125–145
7	BM1824	182–194	184–194	182–194
8	ILSTS028	129–153	137–157	129–159
9	TGLA122	141–163	137–163	137–165
10	TGLA53	155–169	155–165	155–171
11	ILSTS008	175–179	175–179	173–183
12	ETH225	144–148	152–158	144–160
13	TGLA57	77–99	77–99	77–99
14	ETH152	198–206	194–206	194–210
15	TGLA73	119–143	119–143	119–143
16	ILSTS050	148–160	148–160	148–162
17	ILSTS013	119–125	115–123	111–125
18	SPS115	248–258	246–256	242–260

repeat length (MacHugh *et al.* 1997). Clear molecular differences exist between the zebu and taurine genomes that can be used to detect gene flow and introgression between the 2 species (Edwards *et al.* 2000, Edwards *et al.* 2007, Hanotte *et al.* 2000, Hanotte *et al.* 2002, Troy *et al.* 2001). Microsatellite allele size, as well as frequency, is a source of information about ancestral relationship and separate allele length distributions for *Bos indicus* and *Bos taurus* population. When population specific microsatellite alleles are assigned to an individual's parental population, it enables to quantify the amount of introgression from a parental population in a hybrid sample. It is to determine the frequency of those parental population specific alleles in hybrid population (Kumar *et al.* 2003).

The result revealed that Holstein Friesian introgression and Holstein Friesian inheritance levels were independent of each other. The differences in the Holstein Friesian introgression levels of different Karan Fries animals having different inheritance levels could be due to the completely random inheritance pattern, shuffling of autosomal DNA in each individual carrying a unique mixture of DNA from many of their ancestors with a random mixing of chromosomes in each generation, the differing introgression patterns among DNA classes, different potential for introgression among autosomal loci, the interaction effect of introgressing DNA of different sources during introgression and changes in the rate of introgression pattern.

Association of HF introgression with estimated breeding values (EBVs) and expected producing ability (EPA): The mean value of EBV and EPA of the studied Karan Fries cattle population was estimated at 4124.33 ± 391.49 kg and 4200 ± 410.89 kg respectively. It was evident that both EBV and EPA showed increasing trend with increasing HF introgression level. Karan Fries cattle belonging to the 4 introgression groups showed the mean EBVs as 4,036, 4,048, 4,264 and 4,375 kg and EPA values as 4,165, 4,187, 4,363 and 4,464 kg respectively.

Table 3. Karan Fries individuals with their respective Holstein Friesian cattle introgression level (q_{Holstein})

KF Sl.No.	q_{Holstein}	Inh%	F gr.	KF. Sl No	q_{Holstein}	Inh%	F gr.
1	36.16	50.00	F1	43	77.77	64.00	F6
2	57.07	59.40	F7	44	52.23	50.00	F1
3	77.46	54.70	F6	45	39.40	57.42	F8
4	82.28	53.13	F6	46	50.42	56.84	F9
5	50.49	50.00	F1	47	47.46	31.64	F8
6	68.77	59.81	F6	48	61.50	55.47	F8
7	72.78	50.00	F1	49	82.66	37.50	F3
8	65.42	55.00	F8	50	47.82	68.75	F8
9	87.21	50.00	F1	51	33.70	55.00	F10
10	66.93	52.34	F7	52	23.61	62.89	F8
11	43.37	50.00	F1	53	82.60	62.50	F3
12	38.94	62.50	F8	54	38.31	51.00	F9
13	75.08	75.00	F2	55	66.43	50.00	F1
14	52.39	34.40	F6	56	81.89	37.50	F3
15	65.30	50.00	F1	57	52.03	62.30	F9
16	32.10	50.00	F1	58	62.19	63.30	F7
17	70.15	50.00	F1	59	84.48	65.82	F9
18	68.08	59.38	F8	60	82.81	75.00	F3
19	49.13	50.00	F1	61	42.57	65.00	F9
20	33.48	69.92	F8	62	39.14	65.65	F9
21	41.39	50.00	F1	63	54.98	50.00	F1
22	78.48	59.18	F9	64	45.41	62.00	F9
23	25.86	66.00	F8	65	50.11	55.66	F9
24	55.19	75.00	F2	66	42.37	55.66	F9
25	29.42	57.33	F10	67	41.93	61.14	F9
26	52.71	75.00	F2	68	78.16	69.00	F9
27	43.19	55.47	F8	69	64.66	60.16	F8
28	59.24	54.00	F9	70	65.24	64.00	F7
29	70.64	50.00	F1	71	62.42	53.13	F5
30	42.41	57.45	F10	72	34.85	59.29	F10
31	44.60	56.25	F6	73	54.02	56.25	F6
32	56.25	58.00	F10	74	36.43	63.67	F8
33	25.88	26.00	F8	75	51.26	41.41	F7
34	74.69	75.00	F2	76	52.87	71.30	F9
35	15.96	61.00	F7	77	19.77	54.10	F9
36	57.80	68.75	F6	78	45.93	75.00	F2
37	34.77	58.25	F8	79	52.71	68.75	F7
38	51.39	51.56	F6	80	37.75	60.74	F9
39	58.66	75.00	F2	81	35.92	55.66	F9
40	74.17	67.19	F8	82	37.08	57.42	F8
41	48.07	56.00	F9	83	63.56	75.00	F2
42	62.51	75.00	F2	84	66.21	65.50	F10
85	55.24	53.65	F8	127	29.65	54.20	F9
86	72.01	46.30	F9	128	19.12	75.00	F2
87	47.21	48.44	F7	129	73.68	66.41	F7
88	53.81	64.65	F9	130	30.15	53.73	F11
89	83.15	64.26	F9	131	27.73	57.43	F9
90	78.97	75.00	F2	132	68.57	65.00	F9
91	79.73	68.75	F9	133	26.13	55.80	F8
92	50.85	56.25	F9	134	54.72	60.00	F9
93	61.63	65.20	F11	135	35.51	66.40	F5
94	78.83	65.00	F9	136	59.26	69.50	F7
95	69.52	62.50	F3	137	22.76	54.30	F8
96	32.90	64.07	F9	138	29.38	57.43	F8
97	76.46	62.50	F7	139	28.21	62.00	F10
98	54.39	48.44	F7	140	34.57	44.92	F8
99	73.13	68.84	F8	141	67.55	38.00	F9

KF Sl.No.	q_{Holstein}	Inh%	F gr.	KF. Sl No	q_{Holstein}	Inh%	F gr.
100	77.58	68.75	F7	142	58.43	66.00	F9
101	68.74	65.35	F9	143	33.34	50.50	F10
102	66.25	56.84	F9	144	53.81	75.00	F2
103	80.00	75.00	F2	145	48.58	66.50	F11
104	51.46	66.20	F9	146	44.58	56.25	F7
105	29.58	57.83	F11	147	34.59	54.30	F8
106	49.25	50.00	F9	148	19.42	50.00	F2
107	49.24	52.34	F7	149	79.59	54.80	F10
108	36.67	60.55	F8	150	45.71	54.78	F10
109	76.94	63.00	F9	151	32.48	54.30	F8
110	58.96	57.00	F7	152	69.26	62.50	F3
111	26.62	65.63	F8	153	57.84	54.00	F10
112	32.95	60.55	F9	154	50.59	58.10	F10
113	35.67	54.30	F8	155	84.79	56.25	F4
114	84.30	68.75	F7	156	36.28	55.56	F10
115	59.61	54.30	F8	157	52.55	65.63	F5
116	59.54	58.80	F9	158	56.72	53.63	F9
117	45.29	57.00	F7	159	45.50	68.75	F7
118	20.87	57.43	F8	160	42.65	42.70	F10
119	38.60	54.30	F8	161	56.97	60.00	F9
120	25.72	55.00	F10	162	40.92	58.20	F8
121	55.79	63.68	F8	163	43.18	64.90	F10
122	41.29	62.50	F3	164	76.99	71.30	F9
123	63.66	64.85	F9	165	78.24	58.30	F10
124	22.22	59.57	F9	166	34.83	60.25	F10
125	42.29	59.00	F9	167	50.49	50.50	F10
126	69.16	66.30	F10				

q_{Holstein} , HF introgression % in KF; Inh %, HF inheritance % in KF; F gr., Filial group.

The effect on both EBVs and EPA due to increasing HF introgression levels was significant ($P \leq 0.01$). The EBV and EPA increased by 8.02 and 7.37 kg, respectively, with 1% increase in HF introgression level. The significant enhancement of estimated breeding value and estimated producing ability of the Karan fries cattle with increasing HF introgression levels justified that higher exotic introgression levels in crossbreds may help in achieving better production efficiency. But, level of exotic introgression within an individual animal through insemination of exotic germplasm could not be ensured as DNA sharing pattern and DNA-DNA interactions during admixing of genes of 2 populations could not be judged as the introgression of genes is a totally voluntary phenomenon occurring during the crossing over of meiosis. Present study indicated that the genes from HF have been introgressed in the KF cattle very diversely. Although, the result showed positive association between HF introgression levels and the production traits of Karan Fries, the association can be further strengthened to with a larger population involving more number of microsatellite markers.

The regression equations which were developed for EBV and EPA against increasing HF introgression levels were $y = 8.0193x + 3694.8$ (y, EBV of Karan Fries cattle; x, HF introgression levels in Karan Fries cattle) and $y_1 = 7.368x + 3806.1$ (y_1 , EPA of Karan Fries cattle; x, HF introgression

levels in Karan Fries cattle) respectively. From these equations we can state that the EBV and EPA of studied Karan Fries cattle increased by 8.02 kg and 7.37 kg, respectively, with 1% increase in HF introgression level.

Estimation of the genetic admixture proportion can be performed both at individual and population level. In the former case, the fractions of genes in the admixed population are transmitted from either parental population.

In the latter case, a proportion of genes in the genome of a single individual come from a parental population. Both the admixture level i.e. at population level and at individual level was considered here.

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