

Determination of ancestral proportions in synthetic bovine breeds using commonly employed microsatellite markers

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ABSTRACT. The International Society of Animal Genetics (ISAG) has chosen nine microsatellites (international marker set) as a standard that should be included in all cattle parentage studies. They are BM1824, BM2113, INRA023, SPS115, TGLA122, TGLA126, TGLA227, ETH10, and ETH225. We decided to ascertain whether this microsatellite set could be used to determine ancestral proportions in individual animals of synthetic breeds produced by crossing zebu and taurine cattle. Since the genotypes of these markers are routinely available, this would constitute a practical and cost-free method to estimate the ancestry of synthetic breed animals. Genotypes of 100 Gir and 100 Holstein animals were examined for this ISAG marker set. As expected, there were very significant allele frequency differences between the two breeds at most loci. We also typed 20 Girolando animals for which there was complete genealogical information. “Structure” software easily distinguished Holstein and Gir animals based on their microsatellite genotypes; it also attributed the genomic proportion of zebu and taurine of each of the 20

Girolando animals. The proportion of Holstein ancestry was then regressed on the genealogical data; there was a highly significant correlation ($r = 0.84$, $P < 0.0001$). The nine microsatellites that compose the ISAG international marker set were capable of estimating the ancestral Gir and Holstein genomic proportions in individual Girolando animals within narrow confidence limits. This microsatellite set might also be useful for estimating the proportions of taurine and zebu origins in commercial meat products.

Key words: Microsatellites, Ancestry, Taurine, Zebu, Synthetic breed

INTRODUCTION

Bos taurus taurus (European cattle; taurine) and *Bos taurus indicus* (Indian cattle; zebu) are the two subspecies of domesticated cattle. Based on the observation of considerable divergence of microsatellite allelic frequencies between them, MacHugh et al. (1997) estimated that these subspecies diverged more than 600,000 years ago. In the first half of the 20th century, several synthetic breeds were developed by crossing zebu and taurine cattle, as exemplified today in Brazil by the breeds Girolando (Gir x Holstein), Brangus (Nelore x Angus) and Simbrasil (Guzerat x Simmental). All of these breeds, which are claimed to have both high resistance to heat and humidity characteristic of Indian cattle and high productivity characteristic of European cattle, have standard proportions of 3/8 zebu and 5/8 taurine.

The International Society of Animal Genetics (ISAG) has chosen nine microsatellites (international marker set) that should be included in all cattle parentage studies to allow record exchange between laboratories. These are BM1824, BM2113, INRA023, SPS115, TGLA122, TGLA126, TGLA227, ETH10, and ETH225. We decided to ascertain whether this microsatellite set could be used to determine ancestral proportions in individual animals produced from mixed taurine and zebu stocks. Since the genotypes of these markers are routinely available, this would constitute a practical and cost-free method to estimate the ancestry of synthetic breed animals.

MATERIAL AND METHODS

Populations studied

DNA samples from 100 Brazilian Gir animals “puros de origem = pure at origin” and 100 Holstein animals imported from Canada or Europe were examined. We also investigated 20 Girolando animals for which there was complete genealogical information.

DNA analysis

DNA from each individual was independently typed for the nine microsatellites of the international marker set of ISAG. The primer sequence and PCR conditions were those de-

scribed in the webpage of ISAG (<http://www.isag.org.uk/journal/comparisonguide.asp>). Primers were labeled with one of three fluorescent primers: FAM, TAMRA or HEX. The PCR products were analyzed using a MegaBACE 1000 DNA sequencer (GE Healthcare), according to the manufacturer's instructions. Analyses of allele sizes were scored using Genetic Profiler (version 2.2) and Fragment Profiler (version 1.2) software (GE Healthcare).

Statistical analysis

We applied a model-based clustering algorithm by using the Structure software, version 2.1 (Pritchard et al., 2000), which uses a Bayesian algorithm to perform k-means clustering. The genotypes of Gir and Holstein animals were used as parentals. Although zebu cattle also have some taurine ancestry (Kumar et al., 2003), it is relatively small and can be ignored for our purposes. An admixture model with correlated allele frequencies was used. Every run consisted of 50,000 burn-in steps, followed by 250,000 Markov Chain Monte Carlo iterations. Regression analysis was performed with the Statistica software.

RESULTS AND DISCUSSION

As expected, there were very significant allele frequency differences between the two breeds at most loci (Table 1).

Table 1. Allele frequencies of ISAG's international marker set in 100 Holstein animals and 100 Gir animals.

	Allele	Holstein	Gir
BM1824	176	0.006	0.001
	178	0.231	0.064
	180	0.23	0.419
	182	0.147	0.484
	184	0.001	0.005
	188	0.335	0.002
	190	0.042	0.001
	192	0.003	0.024
ETH10	209	0.015	0.367
	211	0.003	0.003
	213	0.072	0.613
	215	0.004	0.002
	217	0.158	0.003
	219	0.511	0.004
	221	0.018	0.001
	223	0.065	0.002
INRA023	225	0.151	0.001
	196	0.003	0.052
	198	0.007	0.065
	200	0.023	0.001
	202	0.164	0.001
	204	0.002	0.012

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Table 1. Continued.

	Allele	Holstein	Gir
	206	0.273	0.046
	208	0.052	0.052
	210	0.166	0.194
	214	0.298	0.534
	216	0.003	0.04
TGLA122	137	0.004	0.17
	139	0.018	0.006
	141	0.016	0.025
	143	0.337	0.195
	145	0.006	0.076
	147	0.004	0.017
	149	0.192	0.044
	151	0.05	0.128
	153	0.01	0.235
	161	0.079	0.044
	163	0.163	0.007
	169	0.006	0.028
	171	0.032	0.011
	173	0.025	0.009
	183	0.059	0.005
TGLA227	77	0.004	0.738
	79	0.002	0.088
	81	0.102	0.139
	83	0.118	0.01
	85	0.002	0.004
	87	0.016	0.003
	89	0.166	0.003
	91	0.193	0.002
	93	0.088	0.01
	97	0.243	0.003
	99	0.018	0.001
	103	0.048	0.001
BM2113	125	0.223	0.002
	127	0.169	0.001
	129	0.003	0.279
	133	0.009	0.001
	135	0.44	0.216
	137	0.094	0.036
	139	0.051	0.188
	141	0.002	0.251
	143	0.002	0.021
	145	0.001	0.005
ETH225	140	0.157	0.184
	142	0.007	0.003
	144	0.025	0.071
	146	0.022	0.001
	148	0.343	0.026
	150	0.361	0.02

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Table 1. Continued.

	Allele	Holstein	Gir
SPS115	152	0.077	0.001
	154	0.002	0.107
	158	0.002	0.574
	160	0.002	0.004
	162	0.001	0.009
	246	0.002	0.237
	248	0.584	0.591
	250	0.002	0.117
	252	0.177	0.001
	254	0.062	0.014
TGLA126	256	0.122	0.036
	258	0.009	0.002
	260	0.031	0.001
	103	0.001	0.009
	105	0.004	0.035
	107	0.003	0.002
	115	0.334	0.084
	117	0.532	0.051
	119	0.013	0.215
	121	0.069	0.019
123	0.035	0.427	
125	0.005	0.14	
129	0.002	0.016	

The Structure software easily distinguished Holstein and Gir animals based on their microsatellite genotypes and also determined the genomic proportion of zebu and taurine for each of the 20 Girolando animals (Figure 1). There was a highly significant correlation between the arc sine of the proportion of Holstein ancestry and the genealogical data (Figure 2, $r = 0.84$, $P < 0.0001$).

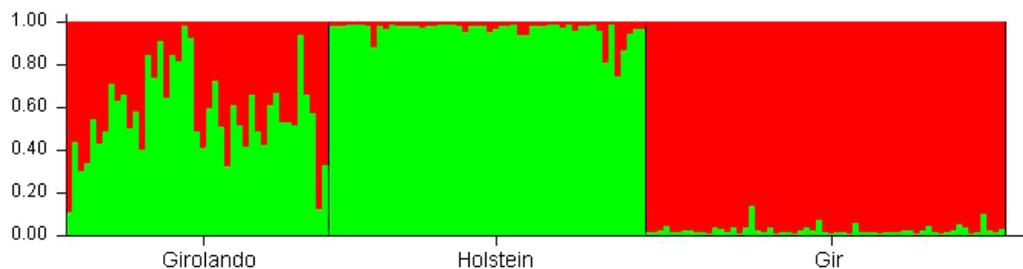


Figure 1. Graph of the estimated Holstein ancestry (in green) of individual animals of the Holstein, Gir and Girolando breeds. Each animal is represented by a thin vertical bar that has green and red segments depending on the relative proportions of Holstein and Gir ancestry, respectively.

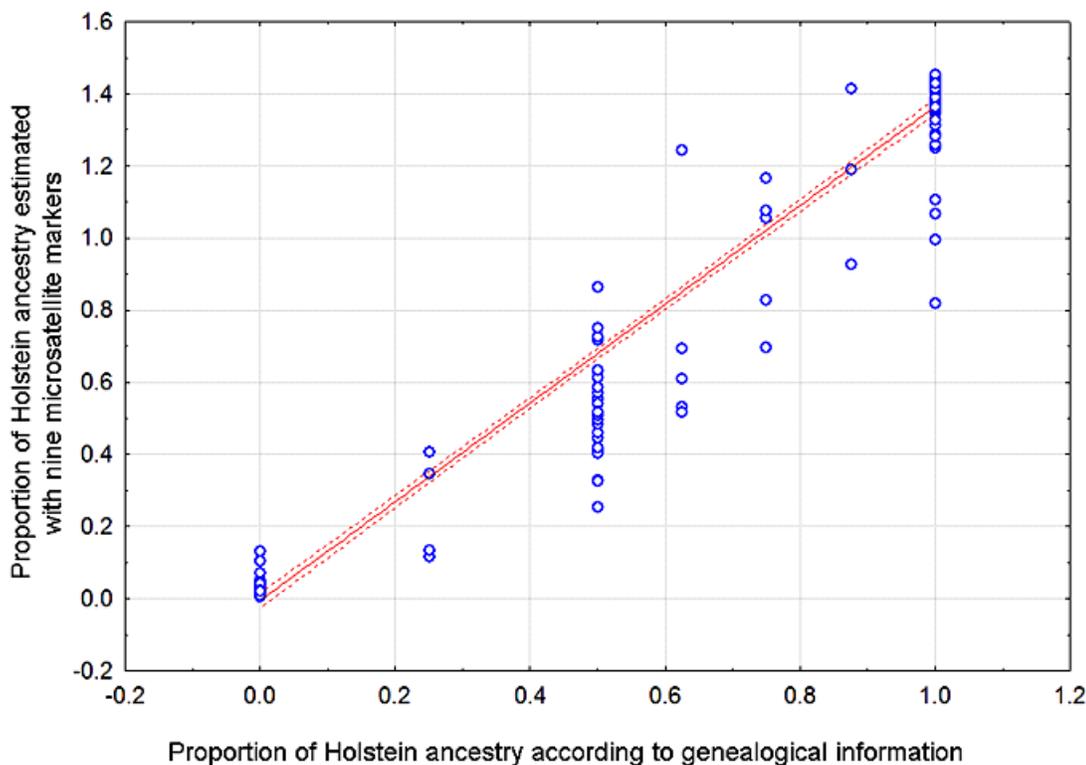


Figure 2. Correlation between the Holstein ancestries of Girolando animals established according to genealogy and the arc sine of the proportion of Holstein ancestry of Girolando animals estimated by the Structure software, using genotypes of the nine loci of ISAG's international marker set. The dotted line indicates the 95% confidence limits of the regression line. The equation of the regression straight line is $y = -0.0055 + 1.37x$ ($P < 0.0001$).

In conclusion, the nine microsatellites that compose ISAG's international marker set can be used to estimate, within narrow confidence limits, the ancestral Gir and Holstein genomic proportions in individual Girolando animals. This same widely available microsatellite set might also be useful to estimate the proportions of taurine and zebu origins in commercial meat products.

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