

# Estimation of the Time of Divergence between Japanese Mishima Island Cattle and Other Cattle Populations Using Microsatellite DNA Markers

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We applied the theory of random genetic drift to determine the divergence history of a closed cattle population over the relatively short timescale of several hundred years. The divergence history of the closed population of Mishima Island cattle, a national natural treasure of Japan, was examined, and the results were compared with historical documents. Inbreeding depression in the isolated population was investigated for body size and fertility. Twenty-one DNA microsatellite markers in Mishima Island cattle and 3 major breeds from the mainland were genotyped. For the mainland breeds, all 21 or 20 markers were segregating. However, nearly half the number of loci (9 of 21) was fixed in the Island cattle. The average number of alleles per locus of Island cattle was markedly lower than that in the mainland breeds. These results support the theory that Island cattle have been isolated for a considerable period of time. The number of generations of isolation was estimated as 14.1–22.6, and the year of divergence was calculated as 1778–1846. In view of these findings, we propose that Island cattle diverged from the mainland population at around 1800 and were isolated for about 200 years. These estimates are in agreement with historical documents showing that divergence occurred between 1672 and 1880. The total inbreeding coefficient of the present population was predicted to be in the range of 0.51–0.60. However, historical reports dated over 100 years do not support changes in fertility, so that there is no evidence for inbreeding depression.

Population genetics can be applied to date divergence events in evolution and archeological science (Zeder et al. 2006). However, estimation using molecular genetic data is mainly applied to problems with very long timescales, typically 10000s of generations (Nei 1987; Zeder et al. 2006). In many cases, divergence in domestic and endangered animals that need conservation occurs over hundreds of years (Takahashi et al. 1998; Solis et al. 2005). It would thus be useful to apply population genetics for dating over shorter timescales and confirm the accuracy of estimation. In this study, we applied the theory of random genetic drift to determine the divergence history of a closed population of cattle. The year of divergence was calculated using molecular genetic data and compared with historical reports.

Mishima is a small island (7.8 km<sup>2</sup>) located in the Japan Sea, 45 km from the main island of Japan (Figure 1). Mishima Island cattle are the oldest breed of native Japanese cattle and were declared a “national natural treasure” in 1928 (male and female photo by Motohashi 1930, Figures 2 and 3). Because Mishima Island cattle were isolated as a closed population, the body shape and size of the original native breed have been maintained. However, the period over which Island cattle have been isolated from those on the mainland remains to be established. Fortunately, historical documents from the 17th century describing the number of cattle on the island were located and compared with our data from genetic analyses. Reduction in the body size and reproduction rates



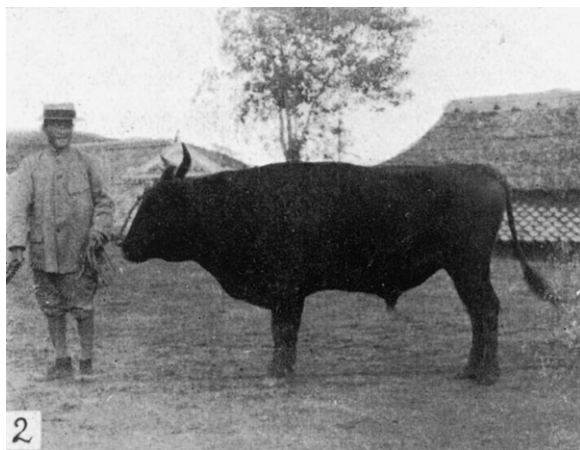
**Figure 1.** Mishima Island.

due to inbreeding are additionally discussed with the aid of reports dating from the 19th century.

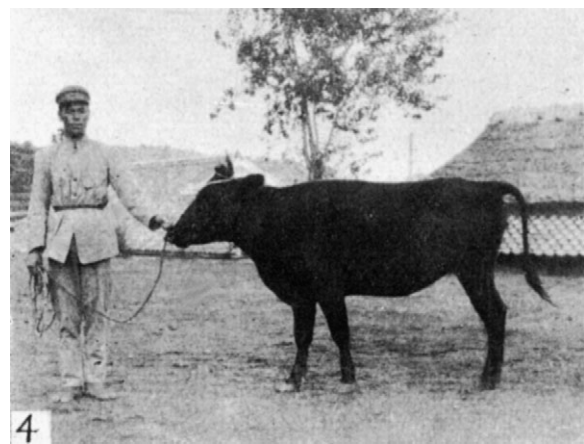
## Material and Methods

We used DNA microsatellite markers from Mishima Island cattle and 3 major Japanese breeds from the mainland, specifically, Japanese Black (Wagyu), Shorthorn (Tankaku), and Brown cattle. A total of 21 markers were typed in 62, 60, 30, and 31 heads of Mishima Island, Black, Shorthorn, and Brown cattle, respectively. Characteristics of the microsatellite markers are shown in Table 1. Markers were located on 17 autosomes, and 4 pairs of markers are on the same chromosome. Linked markers were not used in the estimation of population parameters.

The number of heterozygous genotypes is reduced in a small closed population due to inbreeding (Wright 1969). The 3 major Japanese mainland breeds have much larger population sizes than Mishima Island cattle and, therefore, likely to have experienced much less inbreeding. We assume



**Figure 2.** Island cattle (male) photo by Motohashi (1930).



**Figure 3.** Island cattle (female) photo by Motohashi (1930).

that these major breeds have approximately maintained the heterozygosity of the old Japanese cattle population without gene fixation. This is particularly likely for Black cattle, which comprise the largest population of beef cattle in Japan and are the closest breed to Island cattle. The reduction in heterozygosity per generation is affected by the effective population size. However, changes in the population size over generations lead to inaccuracies in estimation of the effective size. In particular, rapid decrease in population size may cause bottleneck effects, resulting in a swift reduction in heterozygosity. Fortunately, we were able to locate the number of cattle and holder farms on the Island (Table 2). The numbers have varied little from 1739 to 1960 because the cattle were used as working farm animals in the small and stable paddy area of the island. Marked reductions in cattle and farm number were observed after 1960 due to the introduction of agricultural machinery on the farms. The number of cattle decreased significantly to 31 in 1975. However, owing to the recent conservation focus on traditional livestock, the number had increased to 100 by the year 2000. In view of the rapid changes in the number of animals after 1960, we employed the pedigree records of Mishima Island cattle in this study, instead of effective population sizes. An average inbreeding coefficient of 0.3 was estimated using pedigree data after 1960. An estimated effective population size ( $=N_e$ ) of 20 until 1959 and inbreeding coefficient of 0.3 ( $=f_{IS}$ ) after 1960 was assumed in theoretical calculations (Nozawa 1967). Markers were genotyped in the year 2000, and the average generation time was estimated to be 8 years, based on previous reports (Motohashi 1930; Nozawa 1967). Therefore, there were approximately 5 generations of Island cattle from 1960 to 2000.

The heterozygosity of 3 major breeds, Japanese Black, Shorthorn, and Brown cattle, and their weighted average were used to estimate  $H_0$ , the proportion of heterozygotes in the base population. The relationship between the observed heterozygosity in the Island cattle, the time of divergence, and the ancestral heterozygosity is

**Table 1.** Characteristics of the microsatellite

Microsatellite	BTA	Primer sequence (5'–3')	T <sub>a</sub> (°C)	Allele length (bp)	Alleles	References
BM143	6	ACCTGGGAAGCCTCCATATC CTGCAGGCAGATTCITTATCG	60	85–113	9	(1)
BM848	15	TGGTTGGAAGGAAAACTTGG CCTCTGCTCCTCAAGACAC	60	219–229	4	(1)
BM1818	23	AGCTGGGAATATAACCAAAGG AGTGCTTTCAAGGTCCATGC	60	251–275	8	(1)
BM2934	14	CCAATTGTCTTCTAGCTCTTC CTGTTAGTTCTGCCAAAAATCCC	60	74–98	9	(1)
CSSM042	2	GGGAGGTCCTAACTATGGTTGAG ACCCTCACTTCTAACTGCATTGGA	60	172–220	7	(2)
ETH10	5	GTTCAAGACTGGCCCTGCTAACA CCTCCAGCCCCTTTCTCTTCTC	60	205–221	9	(3)
IDVGA37	2	TAAACAGGACAAGTCTTCAGGTG CCTCTCTTCTCTATGCTCAC	60	202–212	6	(4)
ILSTS005	10	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	60	179–181	2	(5)
INRA063	18	ATTTGCACAAGCTAAATCTAACC AAACCACAGAAATGCTTGGAAG	60	172–183	5	(6)
INRABERN172	26	CCACTTCCCTGTATCCTCCT GGTGCTCCCATTGTGTAGAC	60	226–230	3	(7)
INRABERN185	18	CAATCTTGTCTCCCCTATGC CTCCTAAAACTCCCACTA	60	244–250	4	(7)
INRABERN192	7	AGACCTTTACAGCCACCTCTTC GTCCAGAACTGACCAATTTA	60	170–180	4	(7)
MHCII-DR	23	GGACACGTTCTTGCAGATACAACACT GAACTCTCCTTAAGCATACTTGCTC	50	205–227	11	(8)
RM044	29	AAAGTGTGGACATGACTGAAT TTAACAATTAGCAACAGGAATAGAG	50	84–104	8	(9)
AB285206	22	TTGTTTAAAATGTGAAAAGGGG CATTGACAGGCAGGTTCTTT	60	127–155	12	(10)
AB285207	3	CCCCTCCAGTATTCTTGCC TGAGGACAGTAAAACCATCT	60	136–152	8	(10)
AB285208	9	CAGATGTGACTAAAGCAACT GATGGTATTAGGAGGCAAGA	60	99–109	6	(10)
AB285209	19	AGAGCTGGACCGTCCCCTCA ATAGGACAGGGCTGGAGGCT	60	192–194	2	(10)
AB285212	25	TTCCTGGTCGGGACTAACAT GCTGACACGGGTCCACTACA	60	116–140	4	(10)
AB285213	4	CAGTCCACTCTGGTCAGCTTCT CAATGCTGATGGGGTGAAGG	60	195–209	5	(10)
AB285214	5	AGACCCACTTCCAACACCAC CTGCTCCAGACCTGTAGACAAA	60	219–259	5	(10)

BTA, *Bos taurus* autosome; T<sub>a</sub>, annealing temperature; references: (1) Bishop et al. (1994), (2) Barendse et al. (1994), (3) Toldo et al. (1993), (4) Mezzelini et al. (1995), (5) Brezinsky et al. (1993), (6) Vaiman et al. (1994), (7) Saitbekova et al. (1999), (8) Creighton et al. (1992), (9) Kossarek et al. (1995), (10) Kato 2002, registered ID in DNA data bank of Japan.

$$\begin{aligned}
 H_{t+5} &= (1 - f_{IS})(1 - f_{ST})H_0 \\
 &= (1 - f_{IS})(1 - 1/(2N_e))^t H_0 \\
 &= (1 - 0.3)(1 - 1/(2 \times 20))^t H_0, \quad (1)
 \end{aligned}$$

where

$H_{t+5}$ : expected proportion of heterozygous genotypes in the isolated population after divergence at generation  $t + 5$ .  $t + 5$ :  $t$  is the divergent generation until 1959, and 5 represents the number of generations from 1960 to 2000.

$f_{IS}$ : probability that 2 homologous genes in an individual are derived from the same gene in a common ancestor within the isolated population after 1960.

$f_{ST}$ : The probability that 2 homologous genes selected at random from the isolated population are descended from a gene in the isolated population until 1959 (equations using  $f_{IS}$  and  $f_{ST}$  after Crow and Kimura 1970)

We applied this equation to estimate  $t$  for each segregating marker in the populations,

$$t = \log[H_{t+5}/(0.70H_0)]/\log(39/40).$$

For instance, heterozygous proportion of marker IDVGA37 is 0.649 from Black and 0.248 from Island cattle; therefore,  $t$  is 23.9 ( $=\log[0.248/(0.70 \times 0.649)]/\log(39/40)$ ). Average of the  $t$  across markers was used to obtain a single estimate. When  $H_{t+5}$ , the heterozygous

**Table 2.** Number of Island cattle and farms from historical records

Year	Number of cattle	Number of farms
1672	Extinct by rinderpest	
1739	433	255
1800	395	212
1884	580	235
1917	568	246
1928	Designated as a natural treasure	
	568	228
1955	559	Not available
1960	315	214
1965	164	155
1970	59	56
1975	31	25
1980	36	17
1995	104	7
2000	100	7

proportion of Mishima Island cattle, was greater than 70% of  $H_0$  in a major population,  $t$  could not be estimated. These markers were not used in estimation of divergence generations. The divergence year was calculated using estimates for  $t$  and an average generation interval of 8 years. Following the estimation of  $t$ , the total inbreeding coefficient ( $f_{IT}$ ) of Mishima Island cattle was estimated using Equation 2 (Crow and Kimura 1970):

$$1 - f_{IT} = (1 - f_{IS})(1 - f_{ST}) \\ = (1 - 0.3)(1 - 1/(2 \times 20))^t. \quad (2)$$

The terms  $f_{IS}$  and  $f_{ST}$  are similar to those in Equation 1.

## Results

### Genetic Analysis

Nearly half of the number of loci (9 of 21) was fixed as homozygotes in Mishima Island cattle. In contrast, zero or 1 loci were fixed in the other 3 breeds (Table 3). The proportion of heterozygous genotypes and the average number of alleles per locus of Mishima Island cattle are markedly lower than those in the 3 main breeds. Stable numbers and a conservative breeding system facilitated accurate estimation of the effective population size. After determining the number of males and females for reproduction based on data from historical reports (Table 2),

**Table 3.** Genetic properties of markers for 21 loci from 4 breeds

	Island	Black	Shorthorn	Brown
Heterozygosity (%)	22.9	48.8	56.0	61.2
Allele/locus	1.85	4.38	4.33	4.76
Fixed loci	9	1	0	0

Heterozygosity (%), average proportion of heterozygous genotypes per locus; allele/locus, average number of alleles per segregating locus; fixed loci, number of loci that are fixed as homozygote out of 21 marker loci.

**Table 4.** Estimated number of generation ( $t$ ) and the year since divergence of Island cattle using 3 different breeds as ancestral populations

Breed	Number of loci	$t$	Year of divergence
Black	4	22.6	1778
Shorthorn	6	20.8	1793
Brown	9	14.1	1846
Weighed average	5	18.2	1814

Breed, heterozygous proportion ( $H_0$ ) of these breeds and their weighed average are used;  $t$ , number of isolated generation from divergence year to 1959.

we estimated the effective population size as 20 until 1959. The estimated number of generations since a common ancestor and the estimated year of divergence are shown in Table 4. For example, in the case of Black cattle,  $t$  was 22.6, and therefore, the divergence year was 1778 ( $=1959 - 22.6 \times 8$ ). Only a limited number of markers were applied in the analysis because homozygous genotypic markers in Mishima Island cattle and the major breeds, Black, Shorthorn, or Brown, could not be used. In total, only 4–9 markers were used, and all these markers are located on different chromosomes. The estimated year since divergence using  $H_0$  from 3 major breeds and their weighted average varied slightly. The range was 68 years, with the year of divergence ranging from 1778 for Black cattle to 1846 for Brown cattle.

### Comparison with Historical Records

Historical documents reveal that all the Mishima Island cattle became extinct in 1672 due to an outbreak of rinderpest (Table 2). However, cattle were reintroduced from the mainland to Mishima, and the number of cattle and holder farms was recorded in 1739. The Japanese government designated Mishima Island cattle a “national natural treasure” in 1928. We assume that the population had been isolated for at least half a century before this classification. Mishima Island cattle possibly became an isolated population between 1672 and 1880. Using heterozygosity data on microsatellite DNA, we predicted that Island cattle diverged between 1778 (estimated from Black cattle) and 1846 (estimated from Brown cattle). These years are included in the range of 1672–1880 estimated using historical documents. All the divergence years (Table 4) calculated using heterozygous genotypes are in agreement with historical data and support the isolation history of Mishima Island cattle.

### Inbreeding Depression

The range of  $f_{IT}$  is between 0.51 ( $t = 14.1$ ) and 0.60 ( $t = 22.6$ ), using the estimates of the number of generations since divergence from Table 4. The weights of mature female Island cattle were reported as 260–300 (Motohashi 1930), 253 (Ishihara et al. 1955), and 262 kg (Harada et al. 1996). No reduction in weight was observed. Inbreeding depression



**Table 5.** Reproduction rate over 110 years

Year	Female (head)	Produced calves (head)	Reproduction rate (%)
1896	363	175	48.2
1900	460	150	32.6
1905	478	257	53.7
1915	576	202	35.0
1920	516	244	47.3
1925	456	218	47.8
1932	379	158	41.7
1950	574	227	39.5
2000	69	30	43.5
2001	71	27	38.0
2002	71	23	32.4
2003	73	22	30.1
2004	74	32	43.2
2005	74	33	44.6

Reproduction rate (%) = (number of calves/number of female) × 100.

is normally mainly observed in reproduction traits. However, the reproduction rate (calves produced per cow) was not decreased in this case (Table 5). Regression of reproduction rate on year was calculated in 3 sets of data. The first set includes data from year 1896 to 1950 while Island cattle were kept relatively large population. The second set includes data from 2000 to 2005 with small population after having bottleneck effect during 1970s. The third set includes all data from 1896 to 2006. All regressions, reproduction rate on year, from 3 data sets were not significant ( $P > 0.05$ ).

## Discussion

The isolation history of Mishima Island cattle is currently a subject of controversy. One of the critical opinions is that mainland cattle were introduced into Mishima, even after 1900. However, the proportion of heterozygous genotypes and the average number of alleles per locus of Island cattle were markedly lower than those in mainland breeds and are consistent with the Island cattle having been isolated from mainland breeds for a considerable length of time. In view of the relationship between Japanese Black and Mishima Island cattle, a reliable estimate is a divergence year of 1778, with approximately 22.6 generations since divergence. Migration from the mainland in recent years, which is likely to affect the gene frequency in Island cattle, would decrease the gene frequency differences between Island and mainland cattle populations, therefore leading to a reduction in the estimated time of divergence. However, the time of divergence estimated by our group (around 200 years) was relatively long in view of the year of extinction (1672) of the original Island cattle. Both genetic and historical records confirm that an isolation period of around 200 years is reasonable for Mishima Island cattle.

Present-day inbreeding coefficients (between 0.51 and 0.60) were estimated using the estimated time of divergence and using pedigree data after 1960. These values are sig-

nificantly higher than those from ordinary cattle populations. For instance, inbreeding coefficients from a large population were between 0.03 and 0.04 from 3 dairy breeds, Jersey, Ayrshire, and Holstein, in Canada (Sewalem et al. 2006). Fertility, an important character of fitness, may be affected by inbreeding (Falconer 1989). However, several investigators report conflicting results on the effects of inbreeding depression on fertility of cows (Hoeschele 1991; Cassell et al. 2003; Sewalem et al. 2006). The following points should be considered for inbreeding depression: 1) the level of inbreeding relative to a base population, 2) the increment rate of inbreeding per generation, and 3) the number of generations of pedigree records applied in study. The level of inbreeding varies with the population and reduction of genetic variation is affected by population size and selection history (Hill 2000). In addition, inbreeding coefficient and depression do not have a simple linear relationship with fertility. Sewalem et al. (2006) observed a more pronounced effect at an inbreeding coefficient beyond 0.125. It is difficult to find significant inbreeding depression when most animals have a low inbreeding coefficient (say,  $<0.063$ ). In terms of the rate of inbreeding per generation, deleterious genes can be excluded gradually from the population with a slow increase in inbreeding, whereas rapid inbreeding may cause serious problems, with lower fertility and vigor within the population. Although Island cattle are highly inbred due to their long isolation, they do not display significant inbreeding depression. Another such example is Chillingham cattle, which show genetic uniformity (Visscher et al. 2001). It is proposed that cattle have higher tolerance against inbreeding if they are inbred over a pronounced period. However, it is important to remember that neither Mishima Island nor Chillingham cattle are kept for practical usage. Their performance as livestock, for example, production rate and growth rate, is quite different from that of modern breeds.

Inbreeding coefficients, calculated using pedigree records, do not consider history beyond the record of registration. Shortage of pedigree record leads a lower estimated inbreeding coefficient because unknown relationships are ignored. Even if a cow is highly inbred, a low coefficient would be derived with very limited pedigree information. The inbreeding coefficient of Island cattle was estimated as 0.3, using pedigree records after 1960. If the isolation since divergence from ancestral populations is taken into account, then the estimate increases to around 0.6. Marker information facilitates the estimation of inbreeding coefficients beyond the limitations of pedigree information.

We conclude that genetic analysis using DNA microsatellite markers supports the hypothesis that Mishima Island cattle have been isolated for a considerable period of time. Genetic and historic data collectively indicate that Mishima Island cattle diverged from the mainland at around 1800 and were maintained as an isolated population for about 200 years. The total inbreeding coefficient of the present population was estimated to be in the range of 0.51–0.60. However, significant inbreeding depression was not observed over 100 years. Our results suggest that both the

level and rate of inbreeding are important to determine inbreeding depression. Thus, a population genetics approach is practical to date historical events, even over a short timescale.

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## References

- Barendse W, Armitage SM, Kossarek LM, Shalom A, Kirkpatrick BW, Ryan AM, Clayton D, Li L, Neiberghs HL, Zhang N, et al. 1994. A Genetic-linkage map of the bovine genome. *Nat Genet.* 6:227–235.
- Bishop MD, Kappes SM, Keele JW, Stone RT, Sunden SLF, Hawkins GA, Toldo SS, Fries R, Grosz MD, Yoo JY, et al. 1994. A genetic-linkage map for cattle. *Genetics.* 136:619–639.
- Brezinsky L, Kemp SJ, Teale AJ. 1993. ILSTS005—a polymorphic bovine microsatellite. *Anim Genet.* 24:73.
- Cassell BG, Adamec V, Pearson RE. 2003. Material and fetal inbreeding depression for 70-day nonreturn and calving rate in Holsteins and Jerseys. *J Dairy Sci.* 86:2977–2983.
- Creighton P, Eggen A, Fries R, Jordan SA, Hetzel J, Cunningham EP, Humphries P. 1992. Mapping of bovine markers cyp21, prl, and bola drbp1 by genetic-linkage analysis in reference pedigrees. *Genomics.* 14:526–528.
- Crow JF, Kimura M. 1970. An introduction to population genetics theory. New York: Harper and Row Publisher.
- Falconer DS. 1989. Introduction to quantitative genetics. Essex (UK): Longman Scientific and Technical.
- Harada Y, Sakata S, Akimoto I, Fukuhara K, Shinoda T, Hosoi E, Ozawa S. 1996. Experiment for the performance of Mishima cattle. Proceedings of the 8th AAAP Animal Science Congress. 1996 Oct 13–18; Tokyo. Tokyo (Japan). Japanese Society of Zootechnical Science. 356–357.
- Hill WG. 2000. Maintenance of quantitative genetic variation in animal breeding programmes. *Livest Prod Sci.* 63:99–109.
- Hoeschele I. 1991. Additive and nonadditive genetic variance in female fertility of Holsteins. *J Dairy Sci.* 74:1743–1752.
- Ishihara M, Tsuchiya H, Taguchi H. 1955. Comparative studies of the native Japanese cattle (the Mishima cattle) with that of the modern improved Japanese cattle. *Bull Chugoku Nat Agric Exp Stn.* 2:22–54.
- Kato K. 2002. Study on DNA microsatellite polymorphism of Japanese native cattle. Nagoya (Japan): Nagoya University.
- Kossarek LM, Finlay O, Grosse WM, Su X, Armitage SM, Barendse W, Mcgraw RA. 1995. Five bovine dinucleotide repeat polymorphisms—RM040, RM044, RM096, RM153 and RM179. *Anim Genet.* 26:57–58.
- Mezzelani A, Zhang Y, Redaelli L, Castiglioni B, Leone P, Williams JL, Toldo SS, Wigger G, Fries R, Ferretti L. 1995. Chromosomal localization and molecular characterization of 53 cosmid-derived bovine microsatellites. *Mamm Genome.* 6:629–635.
- Motohashi H. 1930. Studies on Mishima ushi, the survived true Nipponese cattle. *Tottori nogaku kwaiho.* 2:83–122.
- Nei M. 1987. Molecular evolutionary genetics. New York: Columbia University Press.
- Nozawa K. 1967. Report of the research group on the native farm animals in Japan and her adjacent localities. *Nagoya Livest Inst Rep.* 2:73–78.
- Saitbekova N, Gaillard C, Obexer-Ruff G, Dolf G. 1999. Genetic diversity in Swiss goat breeds based on microsatellite analysis. *Anim Genet.* 30:36–41.
- Sewalem A, Kistemaker GJ, Miglior F, Van Doormaal BJ. 2006. Analysis of inbreeding and its relationship with functional longevity in Canada dairy cattle. *J Dairy Sci.* 89:2210–2216.
- Solis A, Jugo BM, Meriaux JC, Iriando M, Mazon LI, Aguirre AI, Vicario A, Estomab A. 2005. Genetic diversity within and among four south European native horse breeds based on microsatellite DNA analysis: implications for conservation. *J Hered.* 96:670–678.
- Takahashi H, Nirasawa K, Nagamine Y, Tsudzuki M, Yamamoto Y. 1998. Genetic relationships among Japanese native breeds of chicken based on microsatellite DNA polymorphisms. *J Hered.* 89:543–546.
- Toldo SS, Fries R, Steffen P, Neiberghs HL, Barendse W, Womack JE, Hetzel DJS, Stranzinger G. 1993. Physically mapped, cosmid-derived microsatellite markers as anchor loci on bovine chromosomes. *Mamm Genome.* 4:720–727.
- Vaiman D, Mercier D, Moazamigoudarzi K, Eggen A, Ciampolini R, Lepingle A, Velmala R, Kaukinen J, Varvio S, Martin P, et al. 1994. A set of 99 cattle microsatellites—characterization, synteny mapping, and polymorphism. *Mamm Genome.* 5:288–297.
- Visscher PM, Smith D, Hall SJG, Williams JA. 2001. Viable herd of genetically uniform cattle. *Nature.* 409:303.
- Wright S. 1969. Evolution and the genetics of populations, volume 2: the theory of gene frequencies. Chicago (IL): University of Chicago Press.
- Zeder MA, Emshwiller E, Smith BD, Bradley DG. 2006. Documenting domestication: the interaction of genetics and archaeology. *Trends Genet.* 22:139–155.

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