A viable herd of genetically uniform cattle

Deleterious alleles seem to have been purged in a feral strain of inbred cows.

Inbreeding, which can lead to the loss of genetic variation or the accumulation of deleterious alleles, has been shown to reduce fitness in wild[1], zoo, laboratory[2] and farmed[3] animals. But it has been proposed that when combined with selection, inbreeding may purge deleterious alleles[4]. Here we provide support for this hypothesis in a study of the Chillingham cattle, which shows that this viable herd is almost genetically uniform. The homozygosity of this herd far exceeds that of other cattle[5] and that found in wild populations of other mammalian species[6,7].

This feral herd, which lives in a park in northern England, is thought to have experienced no immigration for at least 300 years (Fig. 1). Despite this genetic isolation, records of calvings and deaths suggest that there has been no drop in fertility or viability[8]. In 1947 the population crashed to five bulls and eight cows; as of 30 October 2000 it numbered 49 individuals. Studies of blood groups and biochemical polymorphisms that represent a small number of genetic loci showed homozygosity for the same alleles at each locus[8].

We obtained tissues from calves, adult cows over 6 years old and bulls that died in 1998–99 ($n=13$). Family relationships and causes of death were unknown; there was no evidence of infectious disease. DNA from the samples was scored for 25 microsatellite markers. These markers are highly polymorphic in cattle[5], with a heterozygosity of typically 70%, and cover 15 of the 29 autosomes. Three markers are located around the bovine major histocompatability complex (MHC) region, where selection might maintain polymorphism[9].

We successfully amplified between 3 and 23 markers for each of the 13 samples, obtaining a total of 225 marker genotypes. (The variation was due to the poor quality of DNA extracted from some hair-root samples.) All samples showed an identical homozygous genotype for 24 of 25 markers. For one marker (*HUJ616* on chromosome 13, amplified or sequenced from 11 samples), nine individuals were heterozygous for the same alleles, and two were homozygous for the same allele, with one allele in common with the heterozygous individuals.

We believe that this single heterozygous marker represents the persistence of ancestral heterozygosity. Assuming 67 generations since 1700, a coefficient of variation of 50% in the population size, and a mean number of sires and dams per generation of 3 and 15, respectively, the effective population size N_e (ref. 10) averages 8.0, and the

Figure 1 Chillingham cattle (*Bos taurus*). This herd, which now comprises 49 animals, lives in isolation in a park in the north of England. The strain has been studied extensively, including by Darwin, because it has remained viable and fertile despite at least 300 years of total inbreeding.

expected proportion of remaining ancestral heterozygosity is $[1-1/(2N_e)]^{67}=0.013$. The probability of segregation at a multiallelic locus with ancestral heterozygosity of 0.70 after 67 generations is approximately $3\times0.70\times[1-1/(2N_e)]^{67}=0.028$ (ref. 11). Thus the probability of finding one heterozygous marker out of 25 markers sampled, using the binomial distribution and assuming unlinked loci, is 0.35, and our data are consistent with the population having been closed for 300 years.

The observed genotype frequencies at the segregating locus are not significantly different from Hardy–Weinberg equilibrium ($P=0.057$, exact test). Re-typing, re-scoring, and sequencing the single marker *HUJ616* gave the same results, making a procedural artefact or a recent mutation unlikely. Heterozygosity could be maintained by selection. Homozygosity was observed at two linked markers on chromosome 13 (9 and 5 animals typed successfully at each). But these markers were 5 and 3 centimorgans from *HUJ616*, respectively, too distant to observe a remaining ancestral heterozygous segment.

For the bovine genome (which has a total map distance of 30 morgans — the expected number of crossovers in the genome during meiosis is 30), with random breeding in isolation for 67 generations, initial complete heterozygosity is predicted to result in 30 heterozygous segments with a mean length of only 1.5 centimorgans[12]. We cannot determine from our data the most likely cause of observed heterozygosity, but

it is consistent with the segregation of alleles at a neutral locus in a small population which has been closed for 300 years.

We believe the continuing viability of the herd shows that deleterious alleles have been purged. No chromosome segments appear heterozygous, apart from a single marker. There is no evidence of heterozygosity near the MHC complex, implying that selection by disease or parasites has not been important at this locus, or perhaps that an optimum haplotype has been fixed. The homozygosity of the Chillingham herd might help to elucidate the bovine genome and the genetics of disease resistance[13].

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