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Session 2: Genetic Diversity of Wild and Domestic Yak: Conservation and Management (9-Apr-2000)

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1. Genetic Variation of Mitochondrial DNA Within Domestic Yak Populations (4-9 Sep 2000)

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Yaks, *Bos grunniens*, are members of the Artiodactyla, family Bovidae, genus *Bos*. Wild yak are first observed at Pleistocene levels of the fossil record. It is generally believed that they, together with the closely related species of *Bos taurus*, *Bos indicus* and *Bison bison*, resulted from a rapid radiation of the genus toward the end of the Miocene. Today domestic yak live a fragile existence in a harsh environment. Their fitness for this environment is vital to their survival and to the millions of pastoralists who depend upon them. Their wild counterparts are further marginalized to the most inhospitable areas of the Qinghai-Tibetan Plateau. Recent introductions of cattle (mainly *B. taurus*), in an attempt to improve stock, may threaten the integrity of the yak genetic make-up with the consequence of reducing fitness with respect to cold temperature and high altitude survival.

Mitochondria DNA (mtDNA) is ideally suited as a tool for studying population genetics because it has the unique features of maternal inheritance, a relatively fast rate of evolution and lack of recombination. Population history is traced back through the maternal line, which excludes the male genome and thus establishes a simple underlying pattern. It is possible to investigate the recent history of domestication due to the fast evolutionary rate of mtDNA. Lack of recombination between the mtDNA of yak and cattle is informative for the study of introgression.

This paper discusses the genetic diversity of Yak expressed in terms of sequence variation found in the displacement loop (D-loop) of mtDNA. A comparison has been made between these values and those found within other domestic species, primarily taurine cattle. An exact time for the domestication of yak is difficult to discern from archaeological data; here we estimate a time based upon a molecular clock. Finally we shall detail the mtDNA introgression observed in yak populations.

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2. Low Level of Cattle Introgression in Yak Populations from Bhutan and China: Evidences from Y-specific Microsatellites and Mitochondrial DNA Markers (4-9 Sep 2000)

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We have used three cattle *Y*-specific microsatellites (Edwards et al. 2000) and one mitochondrial DNA marker to assess the level of cattle introgression into one Bhutanese and five Chinese yak populations sampled from Gannan yaks (GY) in Luqu county, Tianzhu black yak (TBY) in Tianzhu county, and Tianzhu white yaks (TWY) in the Tianzhu White Yak Breeding Farm of Gansu Province, and crossbreeds of domestic with wild yaks (QY) and 3 pure wild yaks (WY) in the Datong Yak Farm of Qinghai Province. One microsatellite, INRA126, proved to be unsuitable in our study with overlapping allelic size between cattle and yak and successful amplification in female yak. At the opposite, microsatellites BM861 and INRA189 were both *Y*-specific in cattle and yak with *Bos taurus*, *B. indicus* and *B. grunniens* diagnostic alleles. Moreover, marker INRA189 was shown to be polymorphic in yak with three alleles. Out of 91 males yak examined, one from the GY population had a cattle taurine *Y* chromosome. Mitochondrial DNA multiplex PCR reactions allowed us to assess rapidly the level of female cattle introgression. We only detected three females yak with a cattle mitochondrial DNA out of a total of 239 animals, one female in the Bhutanese and two females in the Tianzhu white yak populations. Our results indicate that within the five yak populations studied the level of cattle introgression is low.

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3. Genetic Diversity in Bhutanese Yak (*Poephagus Grunneins*) Populations Using Microsatellite Markers (4-9 Sep 2000)

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Eight cattle microsatellite markers were used for genetic analysis of three Bhutanese yak (*Poephagus grunniens*) populations (western, central and eastern). There was substantial genetic variability within yak populations, with average heterozygosity range of 0.644 to 0.680. Neighbor-joining tree constructed from D_s grouped western and central Bhutan yak in one clade ($D_s=0.01$) separate from eastern Bhutan yak ($D_s=0.20$ and $D_s=0.27$). The genetic distances (D_s and F_{st}) between the yak from eastern Bhutan and the other two regions imply that the populations have been separated for at least 4000 years and have exchanged <2 migrants per generation. Based on these results, Bhutanese yak populations are categorized into two types: i) western and central Bhutan yak and ii) eastern Bhutan yak. Implications of these findings on yak conservation and breeding programs are discussed.

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4. Comparison of Microsatellite Variations of Datong Yak With Gannan Yak (4-9 Sep 2000)

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The genetic variations of Datong yak and Gannan yak populations (DTY: *Huanhu subtype* and GNY: *Plateau subtype*) and 3 wild yaks were surveyed using 13 cattle microsatellite markers (recommended by ISAG-FAO). Data of 15 cattle breeds were used as a standard mirror for comparison. Alleles of all 13 loci typed in yak were more or less overlapping with those of cattle, which indicated the flanking region of microsatellite markers between yak and cattle species are conserved. All markers demonstrated polymorphism in the two yak populations studied. The number of alleles ranged from 4 to 8 per locus and the mean number of alleles per locus were 5.462, which is one allele less than that of observed in cattle (6.515). Between the two yak populations, the allele range, most frequent alleles, and alleles of the 13 loci under considered were very similar.

The averages of observed heterozygosities were 0.6385 and 0.6648, and were comparable to means of unbiased heterozygosities of 0.6755 and 0.6511 for two yak populations (GNY and DTY), respectively. None of the loci deviated from H-W-E except loci HEL5 of both yak populations and BM1824 of DTY. Nei's standard Genetic distance between the two yak populations was 0.0324 (D_s), which is less than half of the smallest genetic distance found between two cattle breeds ($D_s = 0.0662$). The result implies that the Datong yak population and the Gannan yak population cannot be further distinguished based on the 13 microsatellites. 42 alleles were found among the 13 loci surveyed in 3 wild yaks, in which, 92.1% were detected in Datong yak population and 84.7% in Gannan yak population. These results obtained demonstrated that the diversities in microsatellite level are richer than in mtDNA level. The richness of genetic diversity within yak population and less genetic distance between yak populations reflected that the genetic diversity are very conservative in the long history of evolution, frequent gene exchanges and random mating system are the major reasons for decreasing the genetic differences of any yak populations and shorten their genetic distance. 13 microsatellite markers are enough in revealing the genetic differences between yak and cattle.

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5. Opportunities for the Improvement of Yak Production With Particular Reference to Genetic Options (4-9 Sep 2000)

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Opportunities for the improvement of yak production are discussed. The non-genetic routes to improvement include nutritional inputs-limited by the availability of supplementary feeds-changes in range management, disease control and increased marketing opportunities.

Genetic routes to improvement, examined in more detail, include selection, crossbreeding and hybridization. Some of the opportunities and difficulties inherent in each are referred to. Particular consideration is given to the potential use of genetic markers, locations on chromosomes identified by molecular techniques. These, if found to be favorably associated with performance traits, or disease resistance, in the yak could be an aid to selection. However, in addition to investment in the molecular procedures, such associations have to be established through recording of performance and parentage identification on a large scale. These pre-requisites are therefore the same as for conventional breeding schemes for yak. In the medium term, such conventional schemes are likely to be less expensive and more certain to be effective, in achieving the desired results, than reliance on molecular techniques. It is concluded that until the molecular technology has advanced much further, to see what it can realistically offer to the improvement in yak productivity, it is important to support long-established practices in order to safeguard a future for domestic yak and for a way of life that it underpins.

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6. Genetic Variations of Yaks in Gansu: Inferred from their Milk Protein Polymorphisms (4-9 Sep 2000)

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Totally 618 yak (*Bos grunniens*) milk samples from 6 Gansu yak populations comparative isolated geographically of Maqu Yak (MQ) from Oula Township of Maqu County, Luqu Yak (LQ) from the Luqu County, Xiahe Yak (XH) from Sangke Township of Xiahe County, Tianzhu White Yak (TZW) from Xidatan Township of Tianzhu County, Tianzhu Black Yak (TZB) from Zhuaxixiulong Township of Tianzhu County and Sunan Yak (SN) from Huangcheng Breeding Sheep Farm in Sunan County, totally 39 milk samples of their F1 hybrids from Datong Breeding Yak Farm in Qinghai Province, and 24 *Bos taurus* milk samples in total from 2 cattle (*Bos taurus*) populations of Qingchuan Yellow Cattle (QC) from Linxia County of Gansu Province and China Holstein Cow (CH) from Dairy Farm of Gansu Agricultural University were taken to determine the genetic variability within populations and genetic differentiation between populations of yaks. Simultaneous phenotyping by conventional discontinuous polyacrylamide gel electrophoresis detected two genotypes (BB and AB) of α -lactalbumin, six

genotypes (AA, AB, AE, BB, BE and EE) of β -lactoglobulin, and two genotypes (AA and AB) of β -casein. The degree of genetic variability within populations estimated by Nei's average heterozygosity for these three milk protein loci of α -La, β -Lg and β -Cn was relatively lower in yaks (0 - 10.62%) than that in cattle (26.61%) and their F1 hybrids (19.65%). The average genetic diversities within yak populations (H_{σ}) and in the total yak population (H_{τ}) were 4.8% and 5.2% respectively. The average genetic diversity between yak populations ($D_{\sigma\tau}$) was 0.44% and the relative magnitude of genetic differentiation among yak populations was 8.4%. The results showed that the genetic variations both within and between yak populations were very limited. It was supposed, therefore, that the close species, such as *Bos taurus*, should be used to richen the genetic diversity of yaks for the sake of improving their productive performances in highland mountain areas and finally for the livelihood of peoples living there. A dendrogram by Unweighted Pair-Group Method with Arithmetic mean (UPGMA) using both Nei's standard distances and Euclidean distances was also given to show the genetic relationships among populations of yaks, cattle and their hybrids. The genetic relationships among populations from tree diagram were somewhat diverged from the yak's historical origins and the possible reasons were also proposed in the paper.

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7. Conserving the Wild Yak on the Tibetan Plateau (4-9 Sep 2000)

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Superbly adapted to the rugged conditions of the highest plateau on earth, the wild yak is a totem animal of Tibet. Endemic to the Tibetan Plateau, wild yaks have declined greatly in distribution and numbers in the last century due to hunting and encroachment on their habitat by pastoralists with livestock. Once numbering in the millions, wild yaks have been extirpated throughout much of their former range. Occupying perhaps a quarter of their original vast range, only an estimated 15,000 wild yaks survive.

Wild yaks are a keystone species and their presence identifies the last, unspoiled ecosystems of Central Asia. Unfortunately, wild yaks are one of the wildlife species on the Tibetan Plateau under the greatest threat today. With their numbers dwindling and habitat being usurped, wild yaks could face extinction in the near future if efforts are not made soon to actively protect them and to conserve and manage their habitat.

Based on research conducted on wild yaks in the Kunlun Mountains of Qinghai Province in 1990 and 1991 and in the Chang Tang Wildlife Reserve of Tibet in 1993 and 1994, the paper provides information on the ecology of the wild yak. The current distribution and status of wild yaks is discussed. Employing new concepts in metapopulation theory, wild yak population models are also presented that help provide a better understanding of wild yak ecology and ecosystem dynamics on the Tibetan Plateau. Models force us to think constructively, help to synthesize available data, and then to explore various implications of management decisions. Finally, recommendations are provided for conserving and managing the remaining herds of wild yak.

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8. Crisis Leading to Decline of Wild Yaks and Issues for their Protection and Utilization (4-9 Sep 2000)

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Due to serious illegal hunting the wild yaks only inhabiting on the Qinghai-Tibetan Plateau have declined dramatically. In later 1980's, there were 20,000 to 40,000 wild yaks found in area of 1.4 million km² around the Kunlun, Arjin and Qilian mountains. However, the golden mining in those regions grown up very rapid from middle 80's and the wild yaks have been to be the meat sources in free charge for the miners since then. It is estimated the loss of numbers of wild yak have been up to by 12,000 heads in the last ten years and 70% of them were hunted and only 30% were died probably during the snow disasters. The concerns for protection of the wild yaks together with the Tibetan antelopes in the regions have been paid more attentions from very recent years by the local governments and people. But the real actions were very difficult to be

supervised due to the limitations of the harsh natural and ecological environments, poorer social, economic and financial supports, and the hard access to the wildlife and their habitats. From the experiences from using the wild yaks to improve the productive performances of domestic ones, the author suggested that the efficient utilization of the wild yaks should be integrated into the protection issues because their significant contribution to and importance in the yak research and development.

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9. Milk Protein Genetic Polymorphism: A Comparison Between Maiwa Yak and Jiulong Yak (4-9 Sep 2000)

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Milk protein genotypes were determined by PAGE using milk samples of 100 Jiulong yaks and 109 Maiwa yaks. Genetic polymorphisms of α_1 -casein, κ -casein, and β -lactoglobulin were found in both breeds. No polymorphism of α -lactalbumin or β -casein was observed. Four variants of α_1 -casein (B, C, D, and E) were detected in both breeds. Two variants each were identified for κ -casein and β -lactoglobulin loci. Significant differences of the genotype distributions of α_1 -casein and κ -casein were discovered between Maiwa yak and Jiulong yak.

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10. Genetic Variants of H Subunit of Lactate Dehydrogenase in the Milk of Maiwa Yak and Jiulong Yak (4-9 Sep 2000)

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The isozymes of lactate dehydrogenase (LDH) in the skim milk of 109 Maiwa yaks and 100 Jiulong yaks were assayed by PAGE. Usually one band (LDH1) was observed for each sample. Four genetic variants of LDH1 were detected: A, B, C, and D according to their mobilities. The D variant was only observed in Jiulong yaks. The C variant was predominant in both breeds. There was significant difference of phenotypic frequency of LDH1 between Maiwa yak and Jiulong yak.

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11. The Immunogenetic Interrelation of Mongolian Yaks With Other Representatives of the Bovinae Subfamily (4-9 Sep 2000)

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The yak is belonged to the Artiodactyla order, Bovidae family, and is the unique representative of the *Poephagus* genus (*P. grunniens* L.). It is known that, by morphological features, yak sharply differs from other representatives of bovines. The purpose of our work was to study interrelation of Mongolian yaks with other representatives of the Bovinae subfamily by

immunogenetic features (blood groups). Initial data were represented with materials of the expedition "Gene pool" by the account of funds of antigens of 47 - 68 designations from 11 - 9 systems of blood groups in a cattle and its relative species, including Mongolian yaks (n=100), and with data of calculation of parameters of the immunogenetic similarity (r) and distance (d) which appropriate to the Euclidean distance (Mashurov and Sukhova 1995). It is carried out 85 comparisons of Mongolian yaks with other representatives of bovines, total number of which was more than 110000. It is established that the least parameters of immunogenetic similarity ($r=0.4588\pm 0.6200$) display in the Mongolian yaks in comparison with zebu, buffaloes, local zebu-shaped cattle of Tadjikistan, hybrids zebu×cattle, as well as with meat (Jiangian, Limujinian, Men-Anjou, light Aquitanian) and combined Kostromskaya breeds, and the most similarity ($r=0.8185\pm 0.7092$) with Buryat and Tadjikistan yaks, and yak x Kalmyk cattle hybrids, as well as with the Gorinian cattle of Belarus and the Simmental meat cattle of Ukraina. Other breeds, by a similarity index with Mongolian yaks, occupy an intermediate position. The location of Mongolian yaks on the hypothetic linear graphics of the Bovindae subfamily is determined. It is characterized by following parameters: $r=0.6539\pm 0.0573$; distances: $d=0.34617$.

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12. Cloning And Sequencing of the 5'-flanking Region of Kappa Casein Gene in Yak (4-9 Sep 2000)

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Primers designed according to the gene sequence of kappa casein in cow (*Bos taurus*) are used to amplify the homologized 5'-flanking sequence in yak (*Bos grunniens*) (436bp). The amplified DNA fragment is cloned and sequenced. Sequence comparison among cattle (*Bos taurus*), sheep (*Ovis aries*), goat (*Capra hircus*) and human (*Homo sapiens*) suggests a potential recognized site of transcription factor "NF-AT^{h.m}" in the yak and cattle sequence (correspondence to -329 to -334nt in yak), which could not be found in sheep and goat. On the other hand, a repeat sequence region belonging to L1PA2 gene family is found inserted in the proximal promoter region of the human kappa-casein gene (from -102 to -210 in the whole insertion from -65 to -374). Another 20bp fragment (from -83 to -102nt in yak sequence) found in the sequences of yak, cattle, sheep and goat is not appeared in the corresponding site in human sequence. The function of these fragments remains unknown. They are supposed to be important regulation elements in gene expression.

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13. Immunogenetic Interrelation of Hybrids (yak x Kalmyk Cattle) With Other Representatives of the Family Bovindae (4-9 Sep 2000)

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The hybridization is one of the most powerful techniques for selection, which, along with a display of heterosis of some economically important features, is accompanied also by sharp change of a genetical structure of hybrid animals with respect to the initial parental forms. The purpose of work was, using data of the immunogenetic analysis of 33 hybrid animals (Tadjik yaks x Kalmyk cattle) and other animals (totally about 110000), to retrace their genetic interrelation, both with the initial parental forms and with 83 representatives of the Bovindae subfamily. It is established that the hybrid animals showed the least similarities ($r=0.5401\pm 0.7193$) with buffaloes, zebu, wisents, three meat breeds (Jiangian, Limujinian, Light Aquitanian), as well as with the local zebu-shaped cattle of Tadjikistan, hybrids zebu×black-variegated breed, Suxunian

cattle, and yaks of Buryatia. The largest similarity ($r=0.8275\pm 0.7826$) in Mongolian yaks was with yaks of Tadjikistan (an initial breed), as well as with Gorinian, black-variegated, Aberdin-Angussian, Holstain-Freezian, and Holland breeds. Similarity of hybrids with another initial breed, Kalmyk one, accounting for 0.7785 ± 6.055 , i.e. was lower than with mentioned above unrelated breeds (Gorinian, Aberdin-Angussian, etc.). Other breeds, including Mongolian yaks ($r=0.7539\pm 0.0707$), occupied an intermediate position by indexes of similarity with yaks hybrids. In hypothetical linear model of the Bovindae subfamily, yak hybrids occupied a point with following parameters: $r=0.7300\pm 0.0645$, and distance $d=0.2700$.

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14. Population Genetic Variations of Hemoglobin (hb) in Yaks, Cattle and their Hybrids (4-9 Sep 2000)

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The method of polyacrylamide gel electrophoresis was used to analyze the Hb polymorphism in six yak populations of Jianzha County of Qinghai Province ($n=43$), and Sunan County ($n=38$), Tianzhu County ($n=63$), Maqu County ($n=70$), Luqu County ($n=36$), Xiahe County ($n=24$) of Gansu Province, two cattle populations from Wuwei city ($n=46$) and Linxia County ($n=22$), and one F1 hybrid population ($n=45$) from Linxia County of Gansu Province. The results showed that the Hb locus of yaks consisted only one genotype (HbAA) for all samples, of cattle from Linxia County had three alleles of Hb^A (0.9091), Hb^B (0.0682) and Hb^C (0.0227) and four genotypes of HbAA (0.9090), HbBB (0.0455), and HbBC (0.0455), and cattle from Wuwei city had three alleles of Hb^A (0.7935), Hb^B (0.1630) and Hb^C (0.0435) and five genotypes of HbAA (0.7174), HbAB (0.1086), HbBB (0.0870), HbBC (0.0435) and HbAC (0.0435). The population of hybrids had two alleles of Hb^A (0.9667), Hb^B (0.0222) and Hb^C (0.0111) and three genotypes of HbAA (0.9333), HbAB (0.0444), and HbAC (0.0222). It was supposed that the Hb locus of pure yak population should be monomorphic but other cattle specific alleles, such as Hb^B and Hb^C, may be introgressed into yak genetics by intensive upgrading.

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15. Study on G6pd of Hybrid Between Yak and Holstein (4-9 Sep 2000)

Y. Wei, Z. Zhang and K. Wu

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16. Genetic Diversity of Blood Isozymes and its Application to Genetics and Breeding in Yaks (4-9 Sep 2000)

Q. Li, Z. Xie

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