Evolution of the $2n = 54$ karyotype of Domestic sheep[1] (*Ovis aries*).

T. D. BUNCH and W. C. FOOTE

International Sheep and Goat Institute
and
Departments of Animal, Dairy and Veterinary Sciences,
Utah State University, Logan, 84322, U.S.A.

Summary

The diploid chromosome number of domestic sheep (*Ovis aries*) evolved either from direct-line ancestral $2n = 54$ stock or from a polyphyletic origin of $2n = 54$, 56 and 58 genetic types. Involvement of any $2n = 56$ or 58 ancestry would have to imply chromosome reduction to the $2n = 54$ diploid type either by genetic swamping of $2n = 54$ inbreeding and /or by an inherent selection toward lower chromosome numbers. The evidence presented here suggests that chromosome evolution in *Ovis* came about by prezygotic selection toward lower chromosome numbers, of $2n = 58$ to 54.

Hybridization between the *Mouflon* ($2n = 54$) and the *Argali* ($2n = 56$) has resulted in selection toward the $2n = 54$ genetic type. The hybrid ewe ($2n = 55$) maintains the greater selective pressure toward reducing the diploid number by usually producing ova with $n = 27$. Of the 18 lambs born thus far from $2n = 55$ parental types, eight were $2n = 54$, nine were $2n = 55$, and one was $2n = 56$. When a $2n = 55$ ram is crossed on a $2n = 54$ ewe the $2n$ type approximates $1/2\ 2n = 54$ and $1/2\ 2n = 55$. The observed alteration in sex ratio (2 females born per male) would contribute to a rapid shift toward lower diploid numbers.

Introduction

Chromosome evolution of *Ovis* involved a progressive reduction in diploid number from $2n = 58$ to $2n = 52$ (EVANS *et al.*, 1973; NADLER *et al.*, 1973a; KOROBITSYNA *et al.*, 1974; BUNCH *et al.*, 1976; and NADLER and BUNCH, 1977). A series of selective fusions of autosomal acrocentrics within the ancestral sheep-goat $2n = 60$ karyotype have formed the 4 pairs of biarmed autosomes common to *Ovis*.

Populations of wild sheep have been partially categorized according to diploid types. Their cytogenetic groupings correspond closely to the natural

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taxonomic groups of wild sheep which comprise the Siberian snow sheep (*O. nivicola*, 2n = 52), North American *Bighorn* and *Thinhorn* sheep (*O. canadensis* and *O. dalli*, 2n = 54), Arkhar/Argali (*O. ammon*, 2n = 56) and the *Urial* (*O. vignei*, 2n = 58) (NADLER *et al.*, 1973*b* and KOROBITSYNA *et al.*, 1974.)

Domestic breeds of sheep typically have a 2n = 54, although translocated variants, T_1, T_2 and T_3 have resulted in chromosome numbers of 52 and 53 (BRUÈRE *et al.*, 1976). The three pairs of biarmed autosomes of 2n = 54 domestic sheep are indistinguishable from those of the 2n = 54 wild sheep (WURSTER and BENIRSCHKE, 1968; BRUERE *et al.*, 1976; and BUNCH *et al.*, 1976). Evidence for the homology of acrocentrics comprising the biarmed autosomes was derived from comparative G-band studies of wild sheep hybrids (*O. vignei*, 2n = 58 × *O. orientalis*, 2n = 54; *O. vignei*, 2n = 58 × *O. aries*, 2n = 54; *O. ammon*, 2n = 56 × *O. musimon*, 2n = 54; *O. musimon*, 2n = 54 × *O. canadensis*, 2n = 54; and *O. musimon*, 2n = 54 × *O. aries*, 2n = 54) (NADLER *et al.*, 1973*a* and BUNCH *et al.*, 1976 and 1977). The 4th pair of biarmed autosomes of *O. nivicola* (NADLER and BUNCH, 1977), however, does not appear to be similar to any of the three types of translocations described by BRUERE *et al.* (1976).

The evolutionary significance of centric fusions resulting in the 2n = 54 karyotype of domestic sheep has not been established previously because of the difficulties involved in bringing together diploid types of differing 2ns. This study was designed to simulate the chromosome dynamics that naturally occur in wild sheep hybrid zones. The same selection principles, however, would appear to be equally applicable to the chromosome reduction or karyotype evolution in domestic Sheep (2n = 54).

Materials and Methods

Wild sheep hybrids with 2ns of 55 were established by mating a kara-tau *Argali* ram (*O. ammon nigrimontana*, 2n = 56) with several *Mouflon* ewes (*O. musimon*, 2n = 54). The F_1 ewes were then backcrossed to the *Argali* ram or bred *inter se* for several generations which produced additional hybrids with 2ns of 54 and 55. Hybrids were placed in three breeding groups each of which includes: 2n = 55 ♂♂ × 55 ♀♀, 2n = 55 ♂♂ × 54 ♀♀ and 2n = 54 ♂♂ and 54 ♀♀.

Data collected over a three-year period allowed interpretations of chromosome dynamics and evolutionary trends based on frequencies of expected versus observed 2ns, ewe fertility and age at first lambing, interval between first and last lamb born of each lambing interval, lambing rates, birth weight of lambs (within 24 h of birth), survivability, and frequency of occurrence of male and female births.

Chromosome preparations were established from 72-hour leucocyte cultures. The 2ns were determined by microscopically examining several intact metaphase spreads.

Results

Mature *Argali-Mouflons* began breeding in late September. Ewe lambs generally reach puberty at 8-9 months of age and breed between October and December. The mature ewes complete lambing in March and within a 17-day period.

Our lambing rate, considering all ewes, was 1.20 (Table 1). This rate increases to 1.50 when limited to ewes bred at the age of 1 year or older. All ewes of $2n = 54$ and 55 were fertile. There was a higher frequency of twinning with $2n = 54$ ewes.

TABLE 1

Diploid chromosome numbers, type of birth and sex of Argali-Mouflon *wild sheep hybrid lambs*
Nombres chromosomiques diploides, type de naissance et sexe des agneaux hybrides Argali-Mouflon

No. of matings	$2n$ of sire	$2n$ of dam	Type of birth		Sex		$2n$ of lambs		
			Single	Twin	M	F	54	55	56
3	54	54	2	1	1	3	4	—	—
7	55	54	3	4	3	8	5	6	—
15	55	55	12	3	7	11	8	9	1

There were approximately twice as many females born as males (Table 1). Males born from $2n = 55 \times 55$ parental crosses had a higher frequency of the $2n = 54$ diploid number (Table 2).

TABLE 2

Nombres chromosomiques diploïdes et sexe des agneaux hybrides Argali-Mouflon
Diploid chromosome number and sex of Argali-Mouflon *wild sheep hybrid lambs.*

No. of matings	$2n$ of sire	$2n$ of dam	$2n$ of lambs					
			male			female		
			54	55	56	54	55	56
3	54	54	1	—	—	3	—	—
7	55	54	1	2	—	4	4	—
15	55	55	5	2	—	3	7	1

Single-birth *Argali-Mouflon* lambs weighed 3.10 ± .2835 (± S.E.) kg and 3.69 ± .3500 kg for females and males, respectively. In contrast, pure *Mouflon* female and male lambs from single births weighed 2.61 ± .1867 and 2.96 ± .2330 kg, respectively. With few exceptions, all lambs were vigorous and survival to 1 year of age was greater than 90 p. cent.

Crosses between $2n = 55$ parental types resulted in lambs with 54, 55 or 56 chromosomes (Tables 1 and 3). The frequency of occurrence of diploid types were one $2n = 56$, nine $2n = 55$ and eight $2n = 54$. The differences between the expected and observed $2ns$ were significant (P < .20, Table 3). The $2n = 55$ heterozygous karyotype comprised 1 unpaired and 2 paired biarmed autosomes, and 2 unpaired and 23 pairs of acrocentric autosomes. The $2n = 56$ karyotype was homozygous for the largest and third to the largest biarmed autosomes of the $2n = 54$ karyotype. The $2n = 54$ was homozygous for all three biarmed chromosomes. Expected and observed frequencies of lambs born from $2n = 54$ ewes bred to $2n = 55$ rams did not differ.

TABLE 3

Nombres chromosomiques théoriques et observés des agneaux hybrides Argali-Mouflon

Expected versus observed diploid types of Argali-Mouflon *wild sheep hybrid lambs*

No. of matings	$2n$ of sire	$2n$ of dam	Expected			Observed		
			54	55	56	54	55	56
3	54	54	4.0	—	—	4.0	—	—
7	55	54	5.5	5.5	—	5.0	6.0	—
15 [1]	55	55	4.5	9.0	4.5	8.0 (*)	9.0	1.0
15 [2]	55	55	1.8	14.4	1.8	8.0 (**)	9.0	1.0

[1] The expected $2n$ frequency of 55×55 crosses would be 1:2:1, if the potential of aneuploid gametes participating in fertilization is discarded, whereas [2] the ratio would be 1:8:1 when considering gametic aneuploid combinations.

(*) $(p < .20 > .05)$.

(**) $(p < .01)$.

Discussion

The 4 pairs of biarmed autosomes common to *Ovis* may have evolved either from a single contiguous population of $2n = 60$ ancestral stocks by a series of centric fusions or independently in different geographic regions. Whatever the mode of evolution, however, specific acrocentrics have evidenced a predisposition to fuse. Generally, the larger acrocentric autosomes have been the first to become involved in biarmed formations. In *Ovis* the order and acrocentric numerical positions (based on the acrocentric positioning in a $2n = 60$ goat karyotype) are metacentric M_1 (acrocentric A_1 and A_7), M_2 (A_4 and A_{14-15}), M_3 (A_3 and A_{12-13}), and M_4 (A_8 and A_{17-18}) (BUNCH et al., 1976 and NADLER and BUNCH, 1977).

According to ZEUNER (1963) the hypothetical prototypes of domesticated sheep were derived from *Mouflon* $2n = 54$ and *Urial* ($2n = 58$) stock. Prior cytogenetic evidence strongly supported the European (*O. musimon*) or Asiatic (*O. orientalis*) *Mouflon* ($2n = 54$) as the most likely progenitor of Domestic Sheep

TABLE 4

Theoretical chromosome combinations of zygotes from heterozygous 2n = 55 wild sheep matings
Arrangement chromosomique théorique des zygotes issus des croisements parentaires à 2n = 55
2n = 55 X
1, 2, 3

	1, 2, 3	1,2	1,3	1	2,3	2	3	0
1, 2, 3 · ·								55
1, 2 · · · ·							55	
1,3 · · · ·						55		
1 · · · · ·				(**) 54	55			
2,3 · · · ·				55	56			
2 · · · · ·			55					
3 · · · · ·		55						
0 · · · · ·	(*) 55							

(*) Euploid zygotic combinations from both aneuploid and euploid gametes (1) 54, (8) 55 and (1) 56.
(**) Euploid zygotic combinations from euploid gametes (1) 54, (2) 55 and (1) 56.

(NADLER et al., 1973a and BUNCH and FOOTE, 1976). The evidence presented herein, however, would suggest that possible genetic contributions by the *Urial* or the *Argali* (*O. ammon*, 2n = 56) in the development of specific breeds of Domestic Sheep cannot be excluded because domestic sheep possess a 2n of 54. Natural selection to the lowest diploid number in hybrids that have heterozygous 2ns would mask the contributing effects of Sheep of higher diploid number.

SAVITZKY et al. (1974) reported (2n = 56) lambs arising from a kazakh domestic breed crossed with a *Urial*. It is unlikely, however, that such hybrids are homozygous and their evolutionary consequences remain to be evaluated. In captive conditions, our crossing of the *Urial* of northeastern Iran to the Chios domestic breed of sheep from Greece also produced F_1 heterozygous (2n = 56) hybrids. However numbers are presently insufficient to evaluate their genetic implications.

The evolutionary significance of the 3 pairs of biarmed autosomes in domestic sheep depends on eigher their impact on the development of reproductive isolation or their role in alterating genetic and morphological characters. CAPANNA et al. (1976) reported spermatic arrest in heterozygous male mice. In contrast, BRUERE (1975) and CHAPHAN et BRUERE (1975) found no evidence of reproductive impairment in domestic sheep heterozygous for T_1, T_2 and T_3 biarmed translocations. Hybrid populations (2n = 55 — 57) of wild sheep of Iran also have displayed normal fertility (VALDEZ et al., 1977). The latter evolutionary consequence yet remains to be evaluated. Hopefully, we will be able to develop our 2n = 56 homozygous stock wherein direct morphological and physiological comparisons can be made to the 2n = 54 genetic type.

Within our captive *Argali-Mouflon* hybrid population, three selective forces are obvious. First, only one of 18 lambs born from 2n = 55 × 55 matings had a

$2n = 56$. Based on the potential random assortment of chromosomes at meiosis, gametes from a hybrid that is heterozygous for 1 unpaired metacentric and 2 acrocentrics (Table 4) could display 1 of 8 possible arrangements. Considering all possible chromosome matchings at fertilization, 64 zygotic combinations are theoretically possible. Realistically, however, only 10 combinations would have balanced chromosomes with a compliment similar to $2ns$ of 54 (homozygous for 3 pairs of biarmed autosomes), 55 (homozygous for the largest and third to the largest biarmed autosomes and heterozygous for 1 unpaired biarmed autosome accompanied by 2 unpaired acrocentric autosomes), and 56 (homozygous for the largest and third to the largest biarmed autosomes).

BRUERE (1975), however, suggested that aneuploid spermatocytes most likely degenerate before sperm maturation. If this occurs in $2n = 55$ male hybrids and is typical during oogenesis of $2n = 55$ female hybrids, then there would be only 4 balanced combinations (Table 4). Such degeneration more than likely accounts for the absence of aneuploid sheep within our controlled study. Therefore, the frequencies of diploid numbers realistically possible from $2n = 55 \times 55$ crosses are: (1) 54, (8) 55 and (1) 56; or (1) 54, (2) 55 and (1) 56, the latter ratio excludes the possibility of aneuploid gametes having a role in fertilization. Of the 17 lambs born thus far from $2n = 55 \times 55$ parental types, eight were $2n = 54$, nine were $2n = 55$, and one was $2n = 56$. The $2n$ type does not seem to shift when a $2n = 55$ ram is crossed on a $2n = 54$ ewe. The ratio approximates $1/2$ $2n = 54$ and $1/2$ $2n = 55$, with five lambs having a $2n$ of 54 and six of 55.

The second selective mechanism noted within our captive hybrid population has been the shifting sex ratio (11 males and 22 females). This ratio alteration (approximately 2 females born for every one male), however is somewhat variable between years of lambing.

The third selective factor we have observed is the age at which the ewes first breed. The *Argali-Mouflon* ewe will usually breed as a lamb and will have her first lamb when she is 12-14 months of age. This surpasses the performance of our captive *Mouflon* ewe lambs. We have no data on fecundal rate of pure argalis, however, wild sheep rarely breed before one year of age (VALDEZ and BUNCH), 1977).

Our results permit the drawing of probable conclusions about the chromosome development of the karyotype of domestic sheep. Since all domestic breeds of sheep commonly have a $2n = 54$, the major prototype was most likely the *Mouflon* (*O. musimon* or *O. orientalis*) or *Mouflon-like* ($2n = 54$) ancestor. The *Urial* ($2n = 58$) and *Argali* ($2n = 56$) may have had a role in domestication, however, for domestic sheep to have a $2n$ of 54, their role in domestication must have occurred at an early time and that genetic swamping by inbreeding of $2n = 54$ sheep has resulted in a stabilized $2n = 54$. The extent of any such influence cannot be determined solely on diploid type, because hybridization with $2n = 54$ stock would automatically bring about a selection or reversion to the lower $2n = 54$ chromosome type.

Résumé

Évolution du caryotype à 2n = 54 du mouton domestique
(Ovis aries)

L'origine du caryotype du mouton domestique (*Ovis aries*) est soit monophylétique (les lignées ancestrales étant à 2n = 54) soit polyphylétique (à partir de types génétiques à 2n = 54, 56 et 58).

Si les ancêtres étaient à 2n = 56 ou 58 la réduction du nombre chromosomique diploïde à 2n = 54 pourrait provenir soit de la généralisation d'un type à 2n = 54 par consanguinité soit d'une tendance sélective à l'adoption d'un nombre chromosomique moins élevé. Les résultats présentés, ici, suggèrent qu'une sélection prézygotique en faveur d'une réduction chromosomique de 2n = 58 à 2n = 54 s'est produite chez *Ovis*.

Lorsque l'on hybride le *Mouflon* (2n = 54) avec l'*Argali* (2n = 56), on remarque, en effet, une sélection en faveur du type génétique à 2n = 54. La femelle hybride (2n = 55) maintient une pression sélective en faveur d'une réduction du nombre diploïde en produisant habituellement des ovules à 2n = 27. Sur les 18 agneaux nés des deux types parentaux à 2n = 55, 8 étaient à 2n = 54, 9 étaient à 2n = 55 et 1 était à 2n = 54. Quand on croise un bélier à 2n = 55 avec une femelle possédant 2n = 54, on obtient approximativement par moitié des 2n = 54 et des 2n = 55.

La déviation du sexe ratio qui est observée (2 femelles pour 1 mâle) contribuerait à un rapide changement vers les nombres chromosomiques diploïdes moins élevés.

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