## G-Band Patterns as Chromosomal Markers, and the Interpretation of Chromosomal Evolution in Wild Sheep (*Ovis*)

Holarctic wild sheep are referable to four groups on the basis of their morphological[1], behavioral[2], and chromosomal characters[3]. A $2n$ of 54 characterized European and Asiatic mouflon sheep ranging from the Mediterranean to northwestern Iran. Urial sheep from northeastern Iran, Turkmen SSR, Tadzhik SSR, and Afghanistan possess $2n = 58$[3,5]. The arkhar/argali group from the USSR and Mongolia have $2n = 56$[3,4]. Finally, North American Bighorn and Dall sheep, of the subgenus *Pachyceros*, have $2n = 54$[6,7]. This latter group includes *Ovis nivicola* from northeastern Siberia, a species as yet unstudied cytologically.

The karyotype of $2n = 58$ sheep contains 1 pair of biarmed autosomes, 54 acrocentric autosomes, a large acrocentric $X$ and a small biarmed $Y$ chromosome. The $2n = 56$ and $2n = 54$ karyotypes include 2 and 3 pairs of biarmed autosomes, respectively, with corresponding reductions in the number of acrocentrics thought to be due to Robertsonian translocations between acrocentric chromosomes. In Bovidae this process proceeded from a primitively high chromosome number ($2n = 58$ in sheep) to a derived lower $2n$[6]. The correspondence of derived diploid numbers ($2n = 54$) in North American wild sheep and in wild mouflon sheep from western Eurasia thus raises the question of chromosomal homologies in these widely separated taxa that are not resolvable by analysis of mitotic chromosomes.

The availability of new techniques for demonstrating specific bands within mammalian chromosomes offers for the first time a simple means of identifying homologous pairs within a karyotype on the basis of morphology[8–11]. These methods for demonstrating Q-, C- and G-bands all yield reproducible criteria for identifying individual chromosomes and recognizing homologous pairs in man and other mammals.

This report describes the mitotic chromosomes and G-band patterns of $2n = 54$ and $2n = 58$ wild sheep of several different New and Old World taxa and meiosis and G-band patterns of their hybrids.

*Materials and methods.* The following specimens were analyzed: 1. desert bighorn (*Ovis canadensis mexicana* Merriam), 2 males from the Kofa Mountains, south of Quartzite, Arizona; 2. European mouflon (*O. musimon* Schreber), 2 females from stock originating in Corsica; 3. Asiatic mouflon (*O. orientalis* Gmelin), 1 male from near Tehran, Iran (35° 41′ N; 51° 34′ E); 4. Urial sheep (*O. vignei* Blyth), 1 male from northwestern Iran (37° 20′ N; 56° 07′ E); 5. hybrid sheep (*O. musimon* × *O. canadensis canadensis* Shaw), 1 $F_1$ male and 1 $F_2$ male; the *O. canadensis* parental stock were captured in northwestern Montana.

Mitotic chromosomes were analyzed from skin biopsies grown in tissues culture by Dr. T. C. Hsu, M. D. Anderson Hospital, Houston, Texas. G-band preparations were made by the technique of Seabright[11] with subsequent Giemsa staining at pH 7.0. Meiotic analysis of the $F_2$ hybrid was performed by the method of Meredith[12].

*Results. Ovis canadensis mexicana* had a $2n = 54$ and the karyotype contained 3 pairs of biarmed and 23 pairs of acrocentric autosomes, a large acrocentric $X$ and a minute biarmed $Y$ chromosome (Figure 1). Comparison of G-bands permitted accurate pairing of the 6 biarmed chromosomes, which are illustrated in Figure 2. G-bands also appeared in certain acrocentric autosomes. The largest acrocentric, considered the $X$ chromosome by most authors, did not exhibit distinct bands; the $Y$ chromosome also lacked distinctive bands. Previous reports of $2n = 54$ in bighorn sheep[6] were based on specimens of *O. canadensis* from the central Rocky Mountains. The presence of an identical karyotype in our new specimens from the southern edge of the species range suggests that bighorns may be found to exhibit chromosomal homogeneity.

The $2n = 54$ karyotype and G-band pattern of the biarmed autosomes from *Ovis musimon* and Iranian *O. orientalis* were identical to those of *O. canadensis mexicana*.

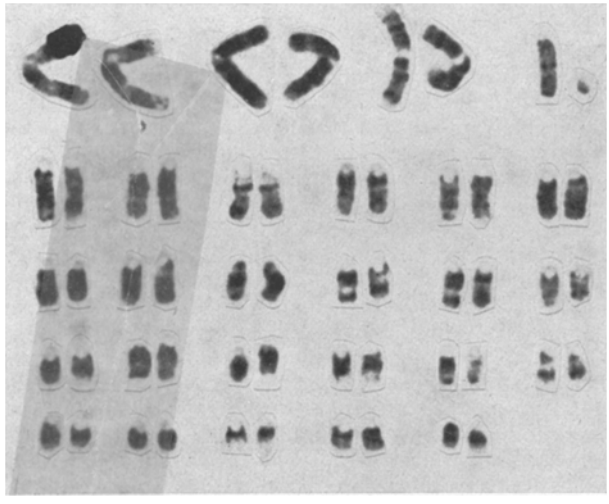

Fig. 1. Karyotype and G-bands of a male *Ovis canadansis mexicana* ($2n = 54$).

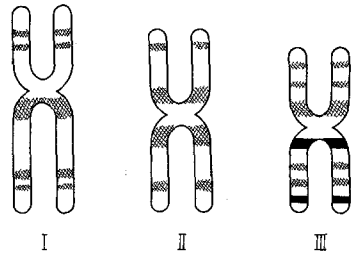


Fig. 2. Schematic representation of G-bands of the biarmed autosomes from $2n = 54$ Old and New World wild sheep.

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[11] M. Seabright, Chromosoma *36*, 204 (1972).
[12] R. Meredith, Chromosoma *26*, 254 (1969).

In contrast, the karyotype of *Ovis vignei* ($2n = 58$) contained only 1 pair of biarmed autosomes (see above); the G-bands of this pair were identical and corresponded to those of the largest pair in $2n = 54$ sheep.

Both the $F_1$ and $F_2$ (Figure 3) *O. musimon* × *O. canadensis* hybrids had a $2n = 54$ and karyotypes and G-bands in the biarmed autosomes indistinguishable from those of *O. musimon* and *O. orientalis*.

Counts of 45 meiotic spreads in diakinesis from the $F_2$ hybrid revealed 27 bivalents including 3 large bivalents that constituted the 3 pairs of biarmed chromosomes and a sex bivalent (Figure 4).

*Discussion.* The presence of identical G band patterns in both members of the 3 biarmed chromosome pairs of $F_1$ and $F_2$ European mouflon × bighorn hybrids strongly suggests that these chromosomes of $2n = 54$ Asiatic and North American sheep are structurally homologous. The homology of these chromosomes in the $F_2$ hybrid is also substantiated by normal bivalent formation in meiosis. Multivalents and univalents, which would be anticipated if mouflon and bighorn karyotypes had experienced random translocations between different acrocentric autosomes to attain grossly similar $2n = 54$ karyotypes, were not observed. The homology of chromosomes comprising the biarmed group is further supported by G-band similarities in $2n = 54$ European and Asiatic mouflon and the American desert sheep.

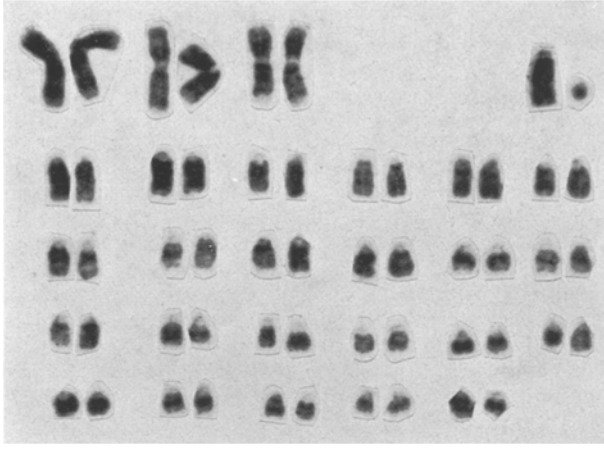

Fig. 3. Karyotype and G-bands of a *O. musimon* × *O. canadensis* $F_2$ male ($2n = 54$).

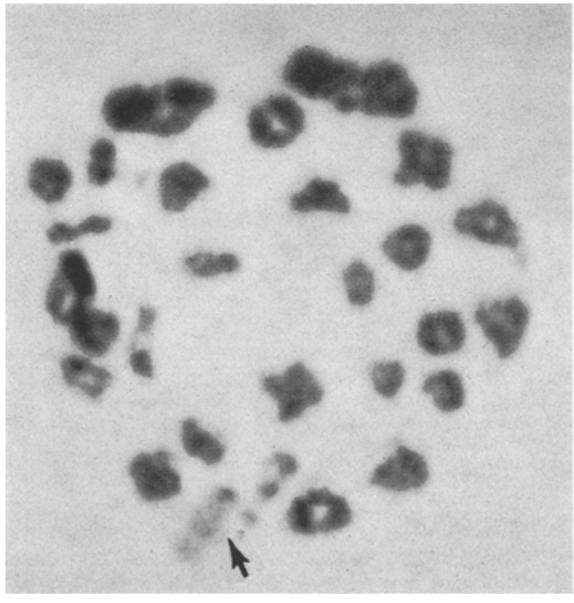

Fig. 4. Diakinesis in a *O. musimon* × *O. canadensis* $F_2$ male with 27 bivalents. The arrow identifies the sex bivalent.

Two alternatives may account for these observations. The first, monophyletic, alternative hypothesizes that all $2n = 54$ sheep evolved from a single common ancestor via Robertsonian centric fusions from $2n = 58$ to $2n = 56$ and finally $2n = 54$. The second, polyphyletic, alternative postulates that the G-band homology exhibited in Asian and North American $2n = 54$ sheep may stem from independently occurring centric fusions that involved the same chromosome pairs. Such non-random fusions have been repeatedly observed in human populations, and in unrelated families account for cases of translocations D-D and D-G group chromosomes[13,14]. Such convergent evolution at the chromosomal level is, if confirmed for wild sheep populations, of great theoretical interest.

The monophyletic alternative would imply that Old World mouflons and New World bighorns are both derived from urial sheep in an early radiation of *Ovis* from Middle Asia[15]. Mouflons spread westward through the Near East to Europe and perhaps Africa, whereas the stock that ultimately gave rise to bighorns spread eastward across the Bering land bridge in the Mid-Pleistocene and reached western North America[16]. The original connection between these now widely separated species must then have been obliterated by extinction of $2n = 54$ sheep across Middle and Central Asia, and their replacement by $2n = 56$ arkhar/argali sheep that evolved more recently from the ancestral urial stock.

Chromosomal convergence would imply that European and Asiatic mouflons were derived from an ancestral urial population isolated somewhere in the Near East or Europe, in which centric fusions resulted eventually in the present $2n = 54$ populations. Independently, and probably earlier, another ancestral urial population was isolated somewhere to the east, in Middle or Central Asia. Centric fusion resulted in a sheep with $2n = 56$ which eventually gave rise to modern arkhar/argali, but which in turn was the source for the amphi-Beringian populations[2]. These ancestral $2n = 56$ sheep, migrating eastward across the Bering land bridge, became isolated from Central Asian $2n = 56$ sheep, and through additional centric fusion, developed into modern Dall and bighorn sheep. For the karyotype of these North American $2n = 54$ sheep to be structurally homologous to that of $2n = 54$ mouflon sheep requires that there be a high probability of fusions occurring between specific acrocentric chromosomes.

A further test of these alternative hypotheses will require comparison of other characters of wild sheep, in particular, morphology, immunology and protein biochemistry[17].

ВЫВОДЫ. Хромосомы и Г-полосы диких баранов из Палеарктика и Неарктика были изучалы. Европейский *Ovis musimon* ($2n = 54$) и Азиатский *O. orientalis* ($2n = 54$)

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имели кариотипы и Г–полосы трех пар две-плечных аутосом одинаковый и этими Северо-Американого *O. canadensis mexicana* ($2n = 54$) В *O. musimon* × *O. canadensis* $\Phi_1$ и $\Phi_2$ гибриды гомологи которые составляли три пары две-плечных аутосом были легко опознаванны и были неразличимые. Изучениэ меиосис в $\Phi_2$ гибриде показало 27 бивалентых. Эволюционное значение гомологическиых две-плечных в баранах через Голарктик было обсужденно.

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## Effet conidiogène sélectif de la thymine chez un mutant «serineless» de *Neurospora crassa*

Les mutants «serineless» (sérine-1 et -2) de *Neurospora crassa* ont un blocage de synthèse au niveau de la transamination du précurseur 3-phosphohydroxypyruvate; ils ne peuvent donc croître qu'en présence de sérine ou de son précurseur aminé en $C_2$, la glycine[1,2]. Nous avions cependant observé que ces mutants sont «leaky», c'est à dire croissent faiblement sur milieu minimal[3]. Le peu de sérine-glycine synthétisé dans ces conditions doit être incorporé dans les protéines et les précurseurs puriques et pyrimidiques des acides nucléiques, répondant ainsi aux besoins immédiats d'un minimum de croissance végétative. En effet, un léger mycélium se développe en profondeur dans le milieu synthétique minimal liquide et ce n'est que sur la zone amaincie du même milieu agarisé, au haut des tubes de cultures, que nous pouvions observer de rares groupes de conidies peu pigmentées (induction par affamement).

La sérine ne fonctionne pas seulement en tant que molécule entière mais aussi comme donatrice de groupe $C_1$ actif pour la méthylation du précurseur pyrimidique phosphorylé (dUMP) en acide thymidylique[4]; elle joue donc un rôle critique dans la synthèse de l'acide désoxyribonucléique (ADN) et par conséquent la division nucléaire. Or, chez *N. crassa*, la question s'est posée de la relation entre divisions nucléaires et conidiogenèse[5]; un certain nombre de figures de mitose avaient été observées dans les chaînettes proconidiennes, ce que confirme une nouvelle étude (en cours avec le Dr. M. CORTAT), basée sur la détection des fuseaux mitotiques. Il devient donc important de préciser le degré de relation entre ces mitoses proconidiennes et le besoin prévisible d'une synthèse accrue d'ADN et, par conséquent, de ses précurseurs critiques, qu'elles doivent entraîner.

Effets conidiogènes versus plasmogènes de précurseurs nucléiques chez le mutant ser-1 de *Neurospora crassa* cultivé 7 j à 25 °C

| Compléments au milieu minimal WM liquide (pH 7,0) | Effets | |
|---|---|---|
| | Poids sec total (mg) | Nombre spécifique de conidies (UO à 660 nm) |
| — | 23 | 0[a] |
| Uracile $10^{-2}M$ | 25 | 240 |
| Thymine $10^{-2}M$ | 28 | 10 800 |
| Sérine $10^{-2}M$ | 194 | 2 450 |
| Sérine $10^{-3}M$ | 107 | 320 |

[a] Petits amas conidiens latéraux déduits.

Il nous est apparu que le mutant sérine-1, amené en milieu minimal à n'investir le peu de sérine synthétisée qu'en croissance végétative, pourrait répondre à un apport supplémentaire de son «produit de luxe», la thymine, par la réaction: davantage d'ADN → mitoses supplémentaires → conidiogenèse sur mycélium restreint. Le correspondant pyrimidique améthylé de la thymine, l'uracile, devrait par contre rester ineffectif. Les résultats préliminaires ayant répondu positivement aux prévisions, nous avons décidé de les présenter ici comme base pour une investigation biochimique plus poussée.

Le mutant «serineless» ser-1 (H 605a, No 118 du FGSC, Arcata, Calif., USA) a été cultivé en milieu synthétique minimal liquide de WESTERGAARD et MITCHELL[6] (WM), distribué à raison de 50 ml dans des flacons Pyrex de 250 ml. La D, L-sérine, la thymine et l'uracile (puriss., Calbiochem) ont été stérilisés avec le milieu dont le pH a été ajusté à la valeur 7,0. L'inoculation a été réalisée avec 4 gouttes d'une légère suspension aqueuse de conidies du mutant récoltées au haut des tubes de cultures sur milieu WM agarisé. Les mycélia ont été récoltés après 7 jours d'incubation à 25 °C en semi-obscurité. Les éventuelles conidies formées ont été séparées et mises en suspension par agitation des mycélia dans de l'eau distillée alcoolisée à 30% et contenant 0,01% du mouillant Tween 80; leur nombre a été estimé par mesure de densité optique à 600 nm en volume connu. Les taux spécifiques de conidiation ont été calculés en unités optiques (UO) rapportées au g de mycélium sec de la culture correspondante; les valeurs fournies ont été corrigées par rapport à celles des cultures témoins sur milieu minimal, considérées comme nulles par l'absence de conidies en surface, vérifiée au microscope. Les poids secs des mycélia ont été mesurés à poids constant (sur silicagel) après dessication à 95 °C.

Nos résultats, groupés dans le Tableau, montrent bien qu'à dose suffisante ($10^{-2}M$), la sérine exerce un double effet de restitution, plasmogène et conidiogène, ramenant ainsi le mutant à un aspect comparable à celui du type sauvage sur milieu minimal. Il est cependant significatif qu'en concentration suboptimale ($10^{-3}M$), la sérine n'est pratiquement plus investie qu'en matériel végétatif prioritaire et que le mutant ser-1 se comporte ainsi, dans ces conditions, en mutant oligo- voire aconidien.

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