Phylogénie moléculaire du Genre Ovis (Mouton et Mouflons), Implications pour la Conservation du Genre et pour l'Origine de l'Espèce Domestique

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THÈSE

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Dedicated:

To my parents and my family in Iran
To my wife Maryam and my children Ahmad and Behzad

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My last remaining task is to acknowledge all those people that have contributed to the work described in this thesis. This is an impossible task, given the many people that have helped to design, sampling, implement, apply, criticize, sponsor and encourage the work. I am going to try anyway, and if your name is not listed, rest assured that my gratitude is not less than for those listed below.

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Abbreviations

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
BP	Before Present
Cyt*b*	Cytochrome *b*
FAO	Food and Agriculture Organization of the United Nations
IUCN	The World Conservation Union (International Union for the Conservation of Nature and Natural Resources)
MB	Bayesian
ML	Maximum Likelihood
mtDNA	Mitochondrial DNA
MYA	Million years ago
NJ	Neighbour Joining
PCR	Polymerase Chain Reaction
YBP	Year before Present

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Chapter 1

Version abrégée en français

1. Version abrégée en français

1.1 Introduction

Cette thèse est structurée en quatre chapitres. Elle commence par une synthèse de la littérature (**Chapitre 2**) qui a pour objectif de faire le point sur la biologie, l'écologie, et la systématique des espèces du genre *Ovis*, ainsi que sur l'histoire de la domestication du mouton. Cette partie présente également le cadre général de la conservation des animaux domestiques. Les chapitres suivants sont constitués d'articles soumis ou acceptés. Le **Chapitre 3** traite de la phylogénie du genre *Ovis*, basée sur l'analyse du Cytochrome *b*. Il retrace l'histoire évolutive de ce groupe et donne des éléments pour résoudre les problèmes taxonomiques existants. Le **Chapitre 4** concerne l'histoire de la domestication du mouton. L'analyse de la diversité génétique nucléaire et mitochondriale du mouton domestique et de ses proches parents sauvage nous permet de retrouver l'ancêtre du mouton domestique et de localiser les centres de domestication. Ces données confrontées aux données archéozoologiques permettent aussi de mieux comprendre le mécanisme de domestication.

Le **Chapitre 5** traitera du problème de la perte des ressources génétiques, en particulier chez la vache, la chèvre et le mouton. L'objectif de cette analyse est de s'interroger sur les lignes de conduite à suivre afin d'avoir une gestion durable des ressources génétiques.

Les premières traces d'animaux domestiques concernent le chien et datent de 14000 à 12000 ans (Turnbull & Reed, 1974). Les premiers animaux domestiques utilisés pour la nourriture qu'ils fournissent sont la chèvre et le mouton, domestiqués il y a environ 11000 ans (Reed, 1984). Il s'en est suivi des milliers d'années de sélection par l'homme de ces animaux domestiques. Plusieurs définitions de la domestication existent dans la littérature. Celle de Price (1984) décrit la domestication comme "le processus par lequel un animal captif s'adapte à l'homme et à l'environnement qu'il fournit". Ainsi, un animal domestique est sélectionné lors de son élevage en captivité, pour répondre aux besoins de l'homme qui contrôle sa nourriture et ses conditions de vie (Diamond, 1999). L'homme a domestiqué

très peu d'espèces. Seules 14 des 148 espèces de mammifères phytophages de plus de 45 kg ont été domestiquées (Diamond, 1999).

Ce processus naturel réalisé par des peuples "primitifs" n'a jamais été observé par l'homme moderne. Le mouton a été entièrement domestiqué pendant la période préhistorique à la fin du Mésolithique (milieu de l'âge de pierre). Les preuves de la domestication initiale du mouton peuvent être divisées en deux catégories par les archéozoologues (Zeder, 2006). Certaines reflètent l'impact de la domestication sur l'évolution de l'homme comme le changement de mode de vie (par exemple sédentarisation). D'autres reflètent les objectifs de l'homme lors de la gestion des populations animales, comme les changements morphologiques (par exemple la sélection de femelles sans cornes). L'archéozoologie n'a pas permis de répondre entièrement à la question de l'ancêtre sauvage du mouton domestique. Le mouflon asiatique (*O. orientalis*), l'Urial (*O. vignei*) et l'Argali (*O. ammon*) sont les trois candidats. Ils sont répartis du Sud-Ouest jusqu'à l'Est de l'Asie.

La famille des Bovidae (Mammalia, Ruminantia) est diversifiée avec 140 espèces classes dans 5 genres (Grubb, 1993). Elle comprend la tribu des Caprini *sensu lato* auquel appartient le genre *Ovis*. Ce genre est l'un des genres de mammifères les plus complexes. Selon les auteurs, des nombres différents d'espèces ont été reconnus, sur des critères biogéographiques, morphologiques, et en fonction du nombre de chromosomes. Nous utiliserons la clasification de Nadler (1973) qui reconnaît sept espèces :

L'Argali (*Ovis ammon*, Linnaeus 1758) qui est le plus grand des moutons sauvages,

Le mouflon asiatique et européen (*O. orientalis*, Gmelin 1774)

L'Urial (*O. vignei*, Blyth 1841)

Le "Bighorn" (*O. canadensis*, Shaw 1804)

Le mouton de Dall ou "Tinhorn" (*O. dalli*, Nelson 1884)

Le "Snow sheep" (*O. nivicola*, Eschscholtz 1829)

Le mouton domestique (*O. aries*, Linnaeus 1758).

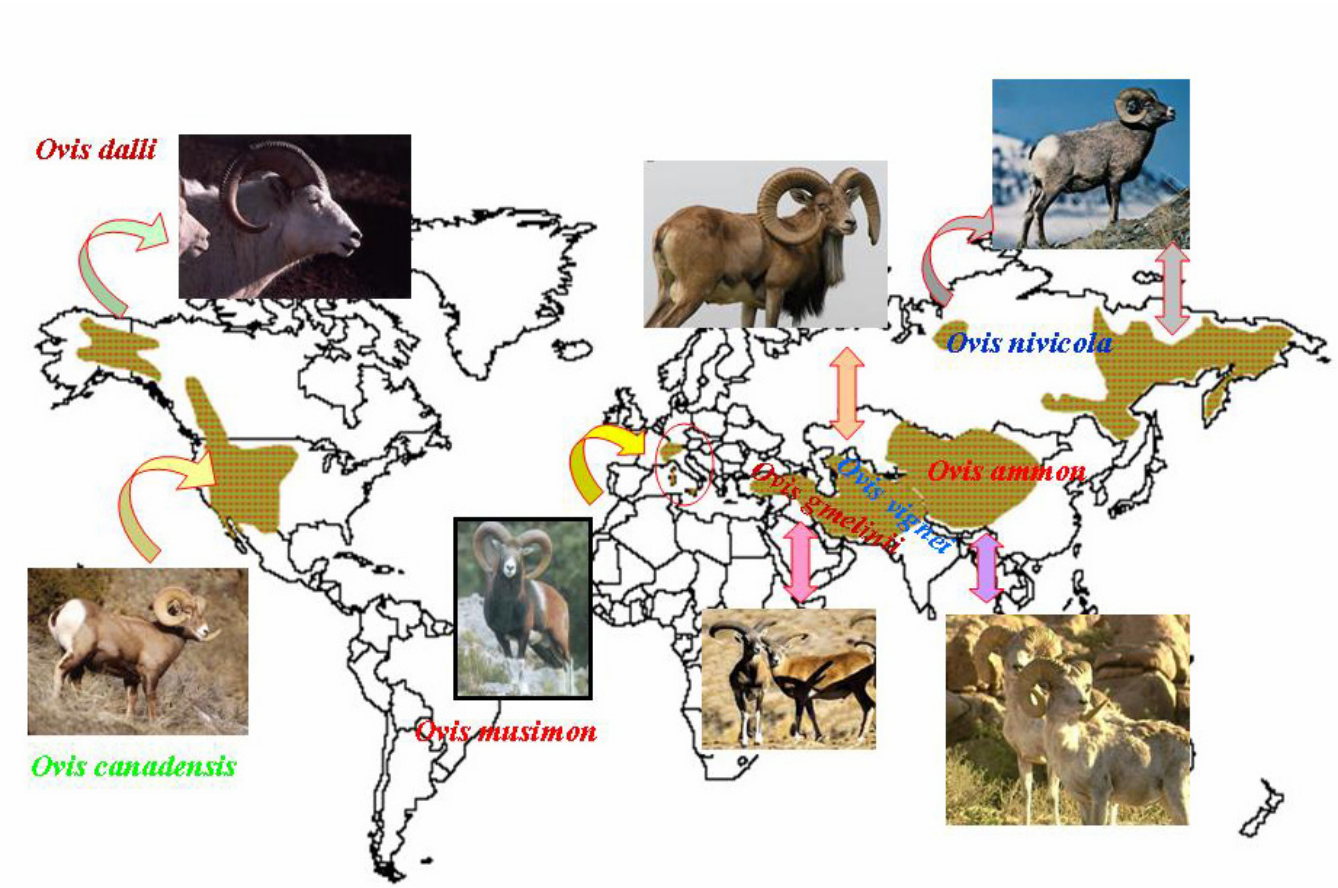
Les analyses génétiques sont utiles pour comprendre les origines de la domestication. Par exemple, la structuration génétique spatiale des espèces domestiques apporte des informations sur les migrations, la comparaison de la diversité des sauvages et des domestiques nous renseigne sur l'origine des animaux domestiques. Pour permettre ce type d'étude, un marqueur moléculaire idéal doit avoir plusieurs caractéristiques. Il doit avoir été suffisamment conservé au cours de l'évolution tout en ayant un taux d'évolution assez rapide pour être variable et structuré dans l'aire de répartition de l'espèce. Nos études

ont essentiellement porté sur le cytochrome *b*. Ce gène mitochondrial répond bien à l'ensemble de ces attentes et, pour ces raisons a été utilisé dans de nombreuses études phylogénétiques en particulier chez les mammifères.

La biodiversité décroît rapidement sous les effets directs et indirects des actions de l'Homme. Un nombre inconnu mais important d'espèces sont déjà éteintes, et de nombreuses autres sont représentées par de petites populations qui présentent un fort risque d'extinction (Frankham, 2003). Approximativement 25% des mammifères, 11% des oiseaux, 20 % des reptiles et 34 % des plantes sont menacés d'extinction d'ici les prochaines décennies (IUCN, 2006). Les actions en biologie de la conservation doivent considérer plusieurs problèmes liés à la génétique, comme la dépression de consanguinité, la perte de diversité génétique, la fragmentation des populations et la réduction des flux géniques. Un outil essentiel pour la gestion et la protection des espèces est également l'identification correcte des populations et des unités taxonomiques.

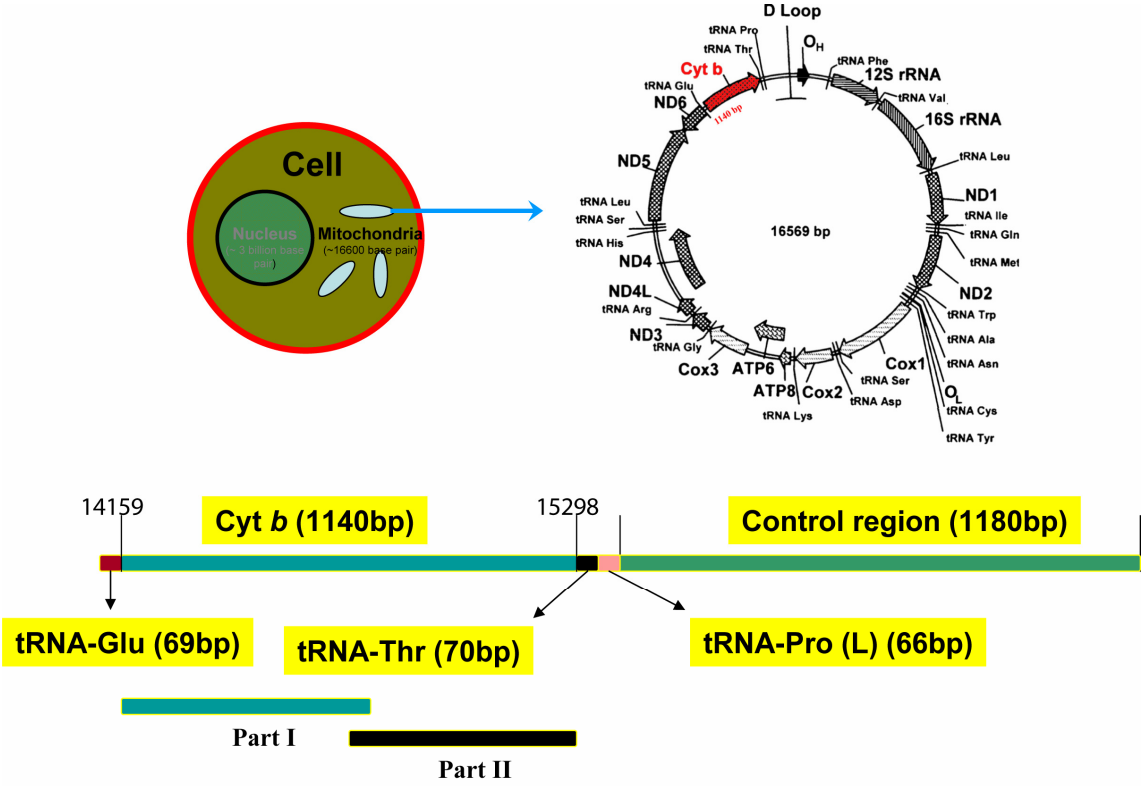
Dans ce contexte, et à propos des animaux domestiques, on prend en compte la notion de ressource génétique. Elle inclut toutes les races des espèces domestiques et les espèces sauvages proches qui ont un intérêt pour l'homme au niveau alimentaire, agronomique, économique, scientifique ou culturel. La conservation des ressources génétiques peut être considérée comme une partie de la génétique de la conservation. Chez les animaux domestiques, la perte des ressources génétiques pourrait être bien plus sérieuse que chez les plantes cultivées, parce que les pools génétiques sont plus réduits et que peu d'espèces sauvages proches existent (Taberlet *et al.*, 2007). Nous en voulons pour preuve que 32% des races d'animaux domestiques dans le monde sont menacées d'extinction ou déjà éteintes, et que ce rythme s'accélère (FAO, 2004). En ce qui concerne plus précisément le mouton, les espèces sauvages proches représentent une source potentielle de matériel génétique qui pourrait servir à améliorer et adapter les races domestiques actuelles à des conditions changeantes (Shackleton & Lovari, 1997).

1.2 Evolution et taxonomies des espèces sauvages du genre *Ovis* (Mammalia, Artiodactyla, Bovidae) : apport de phylogénies moléculaires basées sur l'ADN mitochondrial



Ce chapitre est basé l'article " Evolution and Taxonomy of the Wild Species of the *Ovis* genus (Mammalia, Artiodactyla, Bovidae) Inferred from a Mitochondrial Phylogeny" *de* " Hamid Reza Rezaei, Saeid Naderi, Pierre Taberlet, Hamid Naghash, Delphine Rioux, Amjad Tahir Virk, Mohammad Kaboli, Francois Pompanon" *en révision pour "Molecular Phylogenetics and Evolution".*

La systématique du genre *Ovis* est extrêmement controversée, et plusieurs classifications ont été proposes jusqu'à aujourd'hui. Sept groupes principaux d'*Ovis* sauvages sont distingués sur la base de leur caryotype, de leur morphologie, de leur distribution géographique. L'objectif de cette étude est d'établir une phylogénie de ce genre afin d'en reconstituer l'histoire évolutive et de fournir des éléments permettant de clarifier la classification. Ces phylogénies sont basées sur l'analyse de la séquence de Cyt*b* par des méthodes bayésiennes, de maximum de vraisemblance et de neighbour joining.



Tout d'abord, nous avons réalisé une phylogénie de la sous-famille des Caprinae en analysant 28 espèces. Parmi ces espèces chaque taxon du genre *Ovis* était représenté par deux individus. Cette analyse a permis de confirmer la monophylie du genre *Ovis*. Ensuite,

la phylogénie du genre *Ovis* a été établie à partir de l'analyse de 235 individus échantillonnés sur l'ensemble de l'aire de répartition du genre, et représentant la plupart des sous-espèces connues (15 sur 33). Cette phylogénie permet de clarifier la systématique du genre *Ovis*. Le problème le plus complexe concerne l'Urial et le Mouflon, qui ont été considérés soit comme appartenant à une seule espèce (*Ovis orientalis*) soit comme deux espèces différentes (respectivement *O. orientalis* et *O. vignei*). Ces deux taxons forment deux groupes monophylétiques fortement soutenus (valeurs de bootstrap élevées de 99 sur 100). L'ADN mitochondrial des hybrides entre ces deux taxons les situe dans l'un ou l'autre des groupes parentaux, et ceci quelle que soit leur origine géographique au sein de la zone hybride. La situation des autres taxons est parfaitement claire. Le mouflon européen (*O. musimon*) appartient au clade *d'O. orientalis*. Les autres espèces *O. dalli*, *O. Canadensis*, *O. nivicola* and *O. ammon* sont monophylétiques.

Les données apportées sur les relations phylogénétiques dans la sous-famille des Caprinae et plus précisément entre taxons du genre *Ovis* de permettent de préciser l'histoire évolutive de ce groupe. L'hypothèse d'une origine asiatique du genre *Ovis* est confirmée par nos résultats. Elle aurait été suivie d'une migration vers l'Amérique du Nord via le Nord-est de l'Asie et le Détroit de Béring, et d'une diversification du genre en Eurasie entre 3 et 5 MYA.

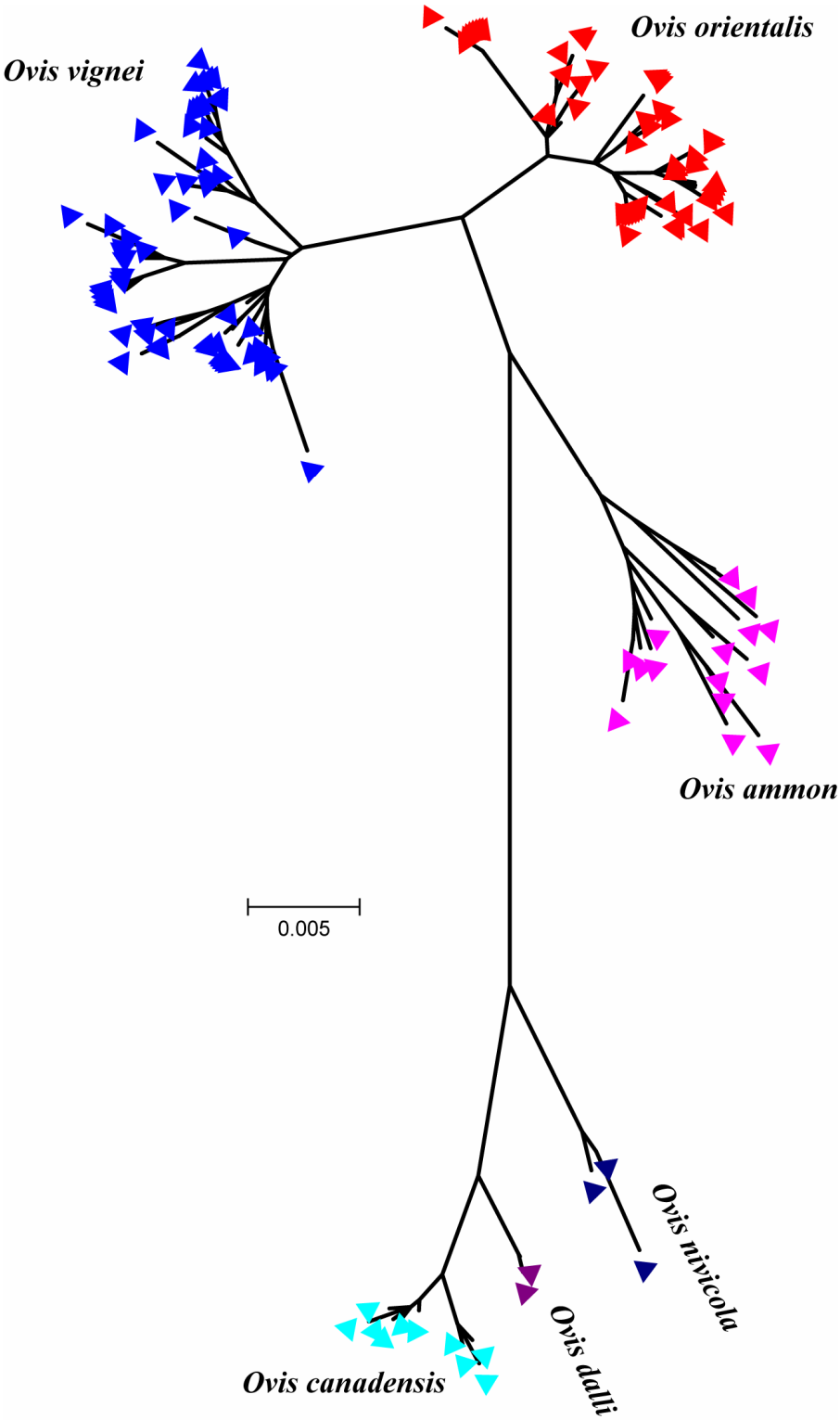
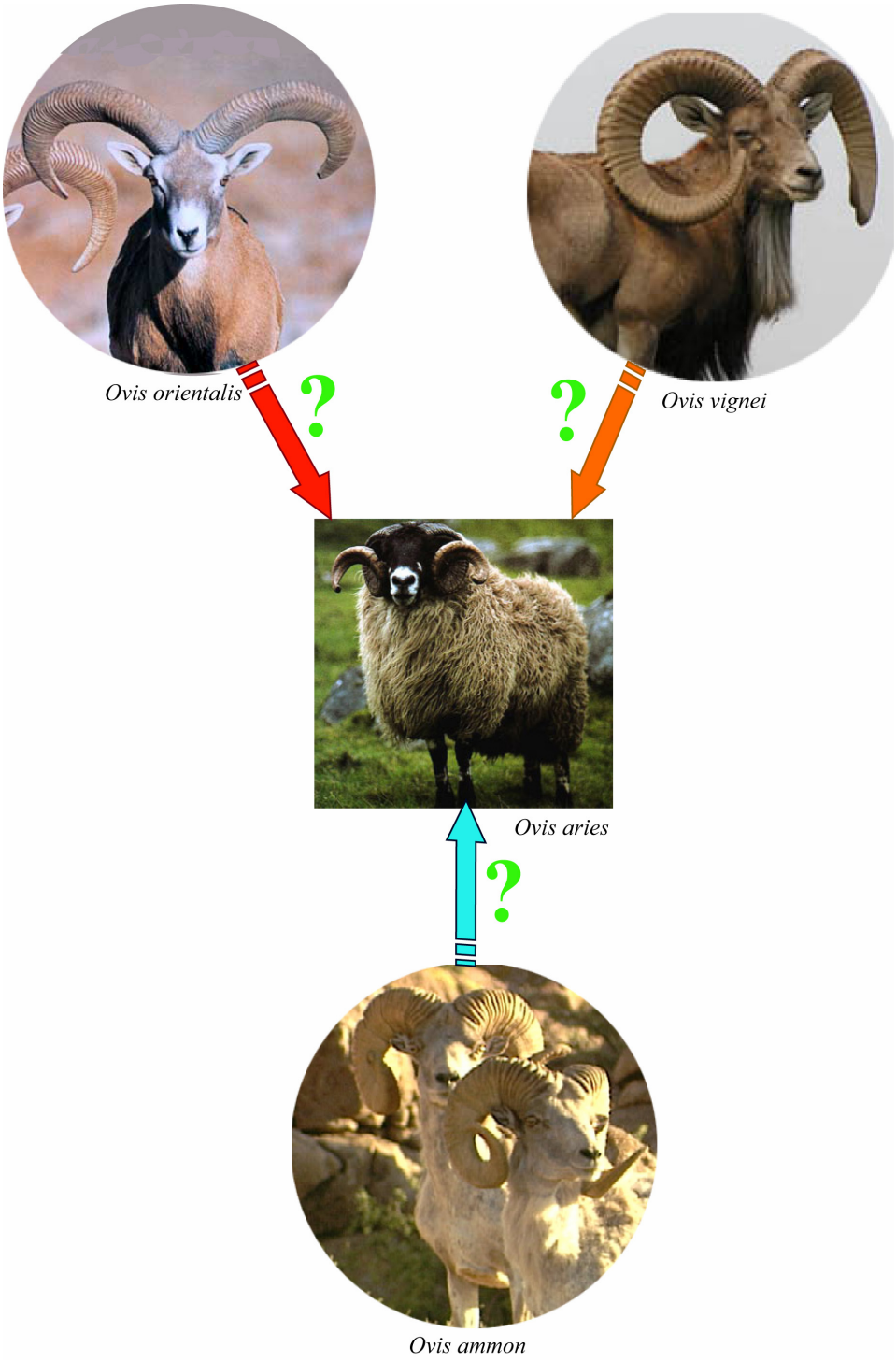


Figure 1-1. Diversité génétique des espèces d'*Ovis* sauvages. Phylogénie obtenu à partir de sequences Cyt*b*.

1.3 L'Origine du Mouton Domestique



Ce chapitre est basé l'article " The Origin of Domestic Sheep " *de* " Hamid-Reza Rezaei, Saeid Naderi, François Pompanon, Marjan Mashkour, Hamid-Reza Naghash, Gordon Luikart, Stéphanie Zundel, Steve Jordan, Deniz Özüt, Aykut Kence, Michael W. Bruford, Jean-Denis Vigne, Pierre Taberlet" *Soumis à xxxxxx (réference)*

Le mouton a été, avec la chèvre l'un des premiers ongulé domestiqué. Il a ensuite été transféré par l'homme sur l'ensemble de la planète. Les premières traces de domestication remontent à 10300 ans cal. B.P. De façon générale, l'histoire de la domestication du mouton est mal connue. L'existence de plusieurs évènements de domestication indépendants est suggérée par la présence de nombreux haplogroupes mitochondriaux fortement divergents chez l'espèce domestique. Mais l'origine du mouton domestique est sujette à controverse. Sur les bases de données archéologiques et génétiques, trois taxons ont été proposés comme étant à l'origine de l'espèce sauvage domestiquée. Il s'agit de l'Argali (*Ovis ammon)*, du Mouflon asiatique (*O. orientalis*) et de l'Urial (*O. vignei*), selon la classification de Nadler *et al.* (Nadler *et al.*, 1973). La localisation du (des) centre(s) de domestication nécessite également d'être précisée. Les données archéologiques indiquent que plusieurs régions auraient été impliquées, et notamment l'Est de l'Anatolie, le Zagros et la vallée de l'Indus.

Dans cette étude, nous avons comparé la diversité génétique du mouton domestique à celle des espèces sauvages proches. Nous nous sommes basés sur l'étude de l'ADN mitochondrial (cytochrome *b*) et de l'ADN nucléaire (fragments répartis dans 12 gènes pour un total de plus de 4000 paires de bases). Nous avons tout d'abord réalisé une analyse phylogénétique. Elle a concerné 130 moutons domestiques et 267 représentants actuels des trois espèces ancestrales possibles. Les individus sauvages ont été échantillonnés dans 55 localités recouvrant la majeure partie de leurs aires de répartition. Les résultats montrent sans ambiguïté que le mouton a été domestiqué à partir d'*Ovis orientalis*. Ensuite, la localisation des individus sauvages qui sont génétiquement les plus proches des domestiques nous apportent des informations sur les lieux de domestication. Le taxon le plus proche du mouton domestique est *O. orientalis gmelini*, qui est localisé dans l'ouest de l'Anatolie et le Nord du Zagros. La domestication se serait donc bien produite dans le Zagros. L'analyse des haplotypes présents chez *O. orientalis anatolica* montre que l'Anatolie Centrale n'a probablement pas été impliquée dans la domestication. *Ovis vignei* et *Ovis ammon* n'ayant pas été domestiqués, l'hypothèse de centres de domestication dans la basse vallée de l'Indus et en Chine n'est pas réaliste.

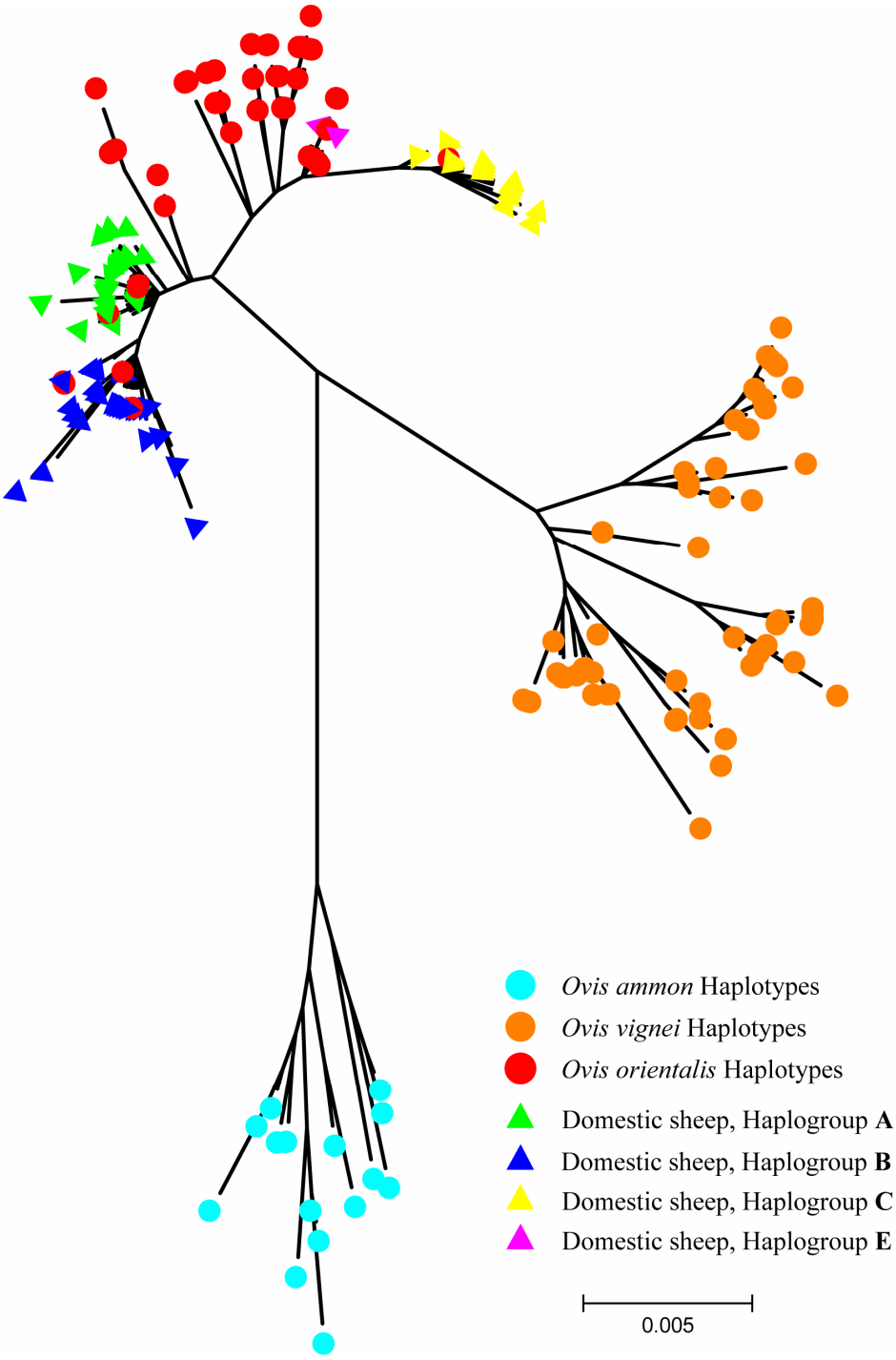
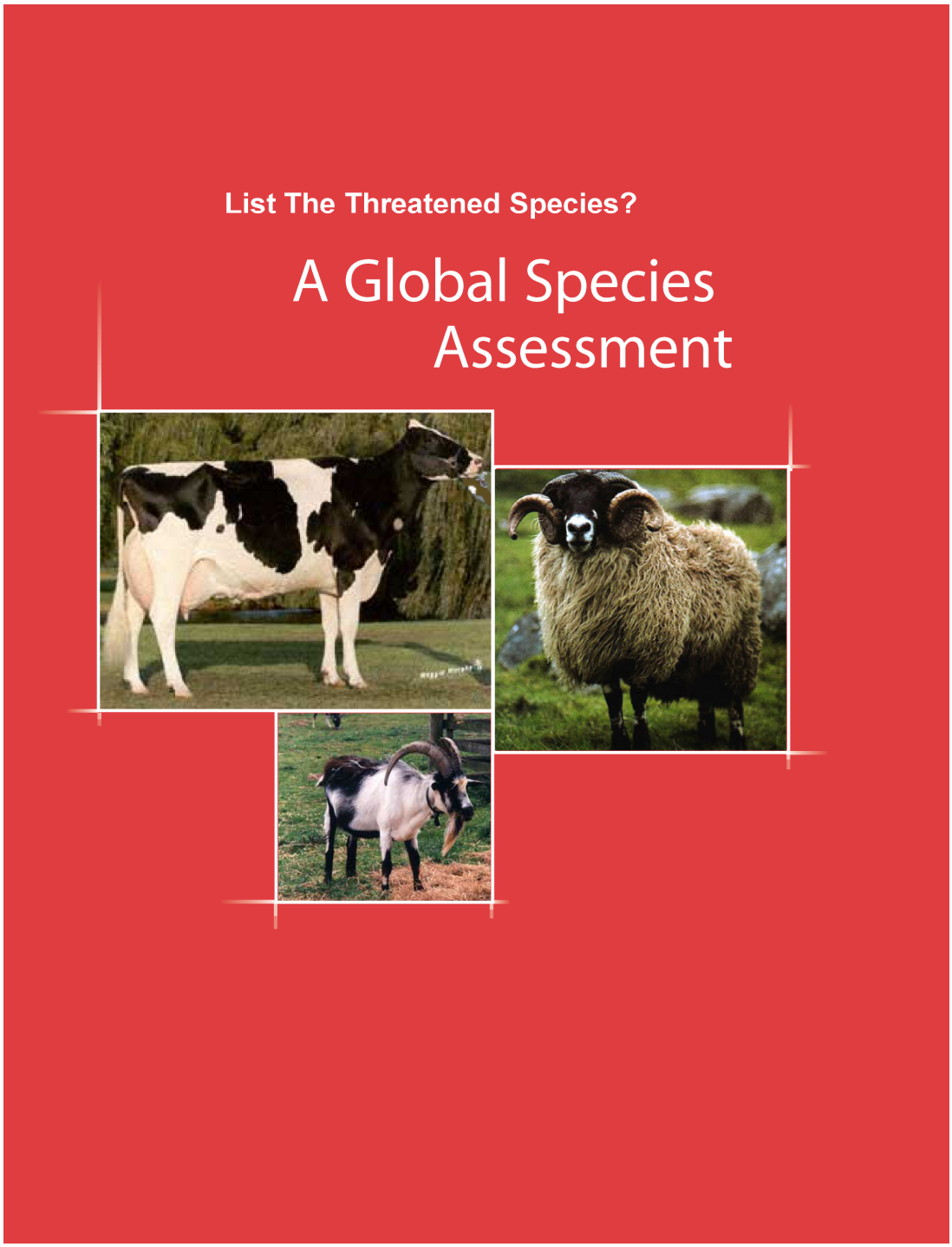


Figure 1-2. Relation phylogénétique entre le mouton et les trois espèces Asiatique du genre *Ovis*.

Chez la chèvre, la domestication a été précédée d'une étape de prédomestication consistant en une gestion initiale durable des populations sauvages conduisant par exemple à la protection contre les prédateurs. Cette étape préliminaire a conduit à une augmentation de la taille efficace de ces populations sauvages à partir desquelles certains animaux ont ensuite été domestiqués, à grande échelle et sans goulot d'étranglement. La signature génétique de cette augmentation d'effectif est toujours visible aujourd'hui sur les chèvres

sauvages (aegagres) qui ont les haplotypes proches de ceux des chèvres domestiques. Nous n'avons pas trouvé de signature d'expansion démographique plus forte chez les mouflons proches génétiquement des moutons domestiques que chez les autres mouflons ou que chez les Urials. Nous ne mettons donc pas en évidence de phase de prédomestication, ce qui ne signifie pas pour autant la présence d'un goulot d'étranglement lors de la domestication. Il apparaît en effet que plus de 200 haplotypes auraient été domestiqués ce qui implique la capture de plusieurs centaines de femelles. Ce résultat est confirmé par la comparaison de la diversité nucléotidique de gènes nucléaire et de la diversité mitochondriale entre sauvages et domestiques. Il apparaît qu'une grande partie de la diversité sauvage a été capturée lors de la domestication, ce qui, tout comme chez la chèvre, n'est pas compatible avec l'existence d'un goulot d'étranglement.

1.4 Les Vaches, les Moutons et les Chèvres Sont-Elles des Espèces Menacées ?



Ce chapitre est basé l'article " **Are Cattle, Sheep, and Goats Endangered Species?** " *de* " **P. Taberlet[1], A. Valentini[2], H. R. Rezaei[1,3], S. Naderi[1,4], F. Pompanon[1], R. Negrini[5], P. Ajmone-Marsan[5,6]** " *publié dans* "***Molecular Ecology***"

Depuis une dizaine de milliers d'années, les fermiers ont géré les vaches, les moutons et les chèvres de façon durable, ce qui a abouti à des cheptels bien adaptés aux conditions locales dans lesquelles ils sont élevés. Il y a environ 200 ans, la situation a commencé à changer dramatiquement avec la montée en puissance du concept de race. Tous les animaux d'une même race ont commencé a être sélectionnés pour exprimer des traits phénotypiques communs. Ainsi, la reproduction entre individus de races différentes a fortement décliné, conduisant à un forte fragmentation des populations initiales.

Depuis quelques décennies, les pressions de sélections ont encore augmenté avec l'objectif d'augmenter la productivité, sans que la préservation de la diversité génétique globale ne soit suffisamment prise en compte. Si l'efficacité des méthodes modernes de sélection a permis une augmentation des rendements de production animale, elle a également eu pour effet une diminution alarmante de la variabilité génétique. De nombreuses races industrielles sont maintenant fortement consanguines avec des tailles efficaces de populations inférieures à 50. Avec le développement de ces races, les éleveurs subissent de plus en plus des pressions économiques les conduisant à abandonner leurs races traditionnelles. Cela a déjà eu pour conséquence la disparition récente d'un grand nombre d'entre elles. Ainsi, les ressources génétiques d'animaux d'élevage tels que la vache, le mouton et la chèvre sont fortement menacées, essentiellement dans les pays développés.

Il nous apparaît donc essentiel de promouvoir des mesures conduisant à une gestion durable des ressources génétiques. Il faut avant tout préserver *in situ* les races menacées. Il est aussi nécessaire de mettre en place des programmes de sélection afin de restaurer la diversité génétique des races industrielles. Enfin, il est indispensable de protéger les espèces sauvages proches des espèces domestiques qui peuvent devenir une ressource génétique très utile.

1.5 Conclusion

Jusqu'à présent, l'évolution du genre *Ovis* a été mal connue. Du point de vue taxonomique, différentes classifications ont été proposées. Elles comprennent de une à sept espèces. Certaines de ces classifications se basent sur des critères morphologiques, d'autres reposent sur des critères chromosomiques et génétiques. La situation est particulièrement complexe dans le centre de l'Iran, où l'on trouve une zone d'hybridation entre le mouflon et l'urial qui produisent des descendants fertiles, bien qu'ils aient des nombres de chromosomes différents. Les arbres phylogénétiques basés sur l'étude de l'ADN mitochondrial montrent que le genre *Ovis* a évolué en deux principaux groupes. Le premier, celui des Pachycériformes, avait été défini sur des critères morphologiques. Il comprend *O. nivicola*, *O. canadensis* et *O. dalli*. Le second groupe, que nous appelons ici les Asiatiformes, est composé de deux ensembles celui des Argaliformes (*O. ammon*) et celui des Moufloniformes (*O. orientalis* et *O. vignei*) qui est paraphylétique. De plus, notre analyse démontre l'appartenance du mouflon européen au clade *O. orientalis*. Ce taxon a donc un rang de sous-espèce (*O. orientalis musimon*). L'absence de fossile de mouflon en Europe avant 5000 ans suggère que ce taxon soit arrivé avec l'homme au néolithique. Cela est confirmé par la proximité génétique entre le mouflon européen et le mouton domestique.

L'homme a domestiqué peu d'espèces d'élevage. Les plus communes actuellement sont la vache, le mouton, la chèvre, le cochon, le cheval et le buffle. La question de l'origine des animaux domestiques est centrale pour comprendre l'histoire de l'humanité. L'origine du mouton domestique est controversée avec trois espèces ancestrales possibles et deux aires de domestication potentielles. C'est cette origine que nous avons recherchée en nous basant sur un échantillonnage important, tant pour les moutons que pour les *Ovis* sauvages. Cet échantillonnage a permis de comparer la diversité génétique des domestiques et des sauvages en analysant la variabilité de l'ADN mitochondrial, et en confrontant nos résultats aux données issues de l'archéologie. Il apparaît clairement que le mouflon asiatique (*Ovis orientalis*) est le seul ancêtre du mouton domestique. De plus, la distribution géographique des haplotypes d'*Ovis orientalis* proches des haplotypes domestiques montre que la domestication s'est produite à l'Est de l'Anatolie et au Nord des monts Zagros, sans aucune participation de la vallée de l'Indus. Il est probable que la

domestication ait débuté par la protection de populations sauvages afin de réduire l'impact des prédateurs.

Les différents haplogroupes trouvés chez le mouton domestique ne proviennent pas de la domestication de différentes sous-espèces comme cela a été évoqué (Hiendleder, 2002). *O. orientalis gmelini* a été la seule sous-espèce impliquée dans des processus de domestication ayant réussi. Il apparaît que la domestication de deux haplogroupes (A et B) s'est produite d'abord dans l'Est de l'Anatolie alors que celle d'autres haplogroupes (C, E et probablement D) a eu lieu ensuite dans le nord du Zagros. Ces deux phénomènes ont été indépendants. Actuellement les moutons domestiques d'Europe occidentale appartiennent aux haplogroupes A et B, alors que les autres groupes sont présents au Moyen-Orient et dans le nord de l'Afrique. Cette distribution géographique suggère que les premiers moutons domestiques ont été amenés en Europe par l'homme en passant par le nord de la mer Méditerranée. Les transferts de moutons par les hommes ont aussi pu contribuer à l'apport d'haplotypes dans les populations naturelles, par l'intermédiaire de domestiques retournant à l'état sauvage. Notre étude montre que des haplotypes trouvés à l'ouest de l'Anatolie chez *O. orientalis anatolica* proviennent de l'est de l'Anatolie et du nord du Zagros.

Le risque d'extinction des espèces peut être réduit par la mise en place d'une gestion des ressources génétiques. Dans ce contexte, la sauvegarde des espèces sauvages proches des domestiques est essentielle puisqu'elles constituent des réserves de diversité génétique pour les espèces domestiques. Ces ressources génétiques sont importantes pour la survie des populations humaines agricoles, mais aussi pour la pérennité des industries agro-alimentaires. Dans les pays en développement, les animaux domestiques représentent des sources de protéines de haute qualité et un facteur de développement économique. L'extinction d'une race ou d'une population signifie la perte de potentialités uniques, généralement gouvernées par de nombreux gènes en interaction, et qui sont le résultat d'interactions complexes entre le génotype et l'environnement. Les extinctions menacent de nombreuses races domestiques dont la variabilité génétique est réduite. Cette variabilité réduite résulte des pressions de sélection imposées par l'homme et par les effets de fondation. Il en résulte des races parfois hautement consanguines, ce qui peut avoir pour conséquence des baisses de fertilité ou de résistance aux maladies. Ces phénomènes sont accentués par le déclin des méthodes d'élevage traditionnelles et le remplacement des races locales par des races industrielles « hautement performantes » dans les pays en développement. Il est donc nécessaire de mettre en place des stratégies de gestion durable

de ces ressources. Ces stratégies doivent prendre en compte les aspects génétiques ainsi que le développement de nouvelles méthodes d'utilisation des ressources. Notamment, la gestion des « petites » populations doit se faire afin d'éviter la consanguinité. Si les espèces domestiques ne sont pas directement menacées du fait de leurs forts effectifs, il est certain que de nombreuses races le sont. L'humanité pourrait perdre dans les prochaines décennies la majeure partie des ressources génétiques qu'elle a lentement sélectionnées depuis plus de 10000 ans.

1.6 Perspectives

Afin d'affiner les arguments génétiques nécessaires pour résoudre les questions de taxonomie, il sera nécessaire d'étudier des gènes nucléaires. L'utilisation de nouveaux marqueurs devrait permettre de tester la validité de nombreuses sous-espèces définies sur la base de critères morphologiques et biogéographiques. De plus, l'utilisation de marqueurs microsatellites ou AFLP permettrait la mesure des flux géniques entre populations afin de comprendre leur structure génétique. Ces résultats auront des implications en génétique de la conservation, en contribuant par exemple à l'identification des populations menacées. Il sera nécessaire de protéger ces populations qui constituent une ressource génétique pour le mouton domestique.

Si l'on considère l'histoire de la domestication du mouton, de nouvelles études sur des sites archéologiques devraient permettre la collecte d'échantillons anciens qu'il sera possible de comparer aux échantillons actuels, issus d'individus sauvages et domestiques. Des scans génomiques devraient permettre de détecter les mutations différenciant ces échantillons, et ainsi d'identifier les gènes impliqués dans le processus de domestication.

Chapter 2

Introduction

2. Introduction

2.1 Domestication

The oldest populations that are assigned to the human (hominid) family lived some 14 million years ago (MYA), differentiated little but sufficiently from contemporaneous small apes to be known as having the potential to evolve into modern humans. The first evidence of a domestic animal, a dog, is dated between 14000 and 12000 years before present (YBP) (Turnbull & Reed, 1974), and the earliest known domestic food animals were goat and sheep about 11000 YBP (Reed, 1984). Thus hominids (humans and their humanlike ancestors), survived for 99.9 percent of their known history without domestic animals or cultivated crops. These 14 million years of hominid history has been pre-eminently a period of invention and use of secondary energy traps that served slowly at first, but with a quickening pace as time passed, to divert increasing amounts of energy through the hominid population thus increasing its numbers and biomass. The domestication of animals is thought to have been the key step in the development of civilization (Diamond, 1999).

Humans and the different kinds of domestic animals and plants are excellent examples of mutualism and thus of mutual secondary energy traps. The human protects, feeds, and cares for the domesticate in numerous ways and is thus a secondary energy trap for the non-human partner; the rewards to the human may be in terms of meat, skins with pelages, leather, milk, fiber, draft power, glue, fertilizer, prestige, and/or companionship. Humans are not the only domesticators; several kinds of ants keep other insects, all suckers of plant juices, as domestic stock from which the ants receive sweet and nutritious droplets. The ants protect, move, build shelters for, and in general care for their "cows" with remarkable success. Other kinds of ants are extremely successful horticulturalists, who gather a variety of organic foodstuffs for their underground crops of fungi (Reed, 1984).

Thousands of years of selective breeding of domestic species have led to marked phenotypic changes and genetic adaptation to various environmental conditions. Therefore, populations of domestic animals have a rich collection of mutations that affect phenotypic traits. Some of these traits, have a simple monogenic basis, but most, such as coat color (Maudet & Taberlet, 2002), growth, fertility and behaviour, are complex multi-factorial

characters (Andersson & Georges, 2004). The advantages of domestic animals will become increasingly important as we move into the post-genomic era. Despite the fact that we now know the complete or near-complete genome sequences of several organisms, our knowledge of the genes that underlie phenotypic differences within and among species is rudimentary.

2.1.1 What Is a Domestic Animal?

It is difficult to define a wild and a domestic animal. A wild animal is usually thought of as one that is fearful of humans and runs away if it can. However, this fear of humans is in itself a behavioural pattern that has learned from experience of human predation over countless generations. A "wild" animal that has no contact with humans has no fear of them and can quickly be exterminated. In the category of "domestic animals" those whose breeding is or can be controlled by humans are included, but most animals in zoos and circuses and many animals (various rodents and primates) in experimental research centers are excluded because they have not truly been brought "into the house".

Several definitions of domestication can be found in the literature. Among them, Price (1984) defined domestication as "the process by which captive animals adapt to man and the environment he provides"(Price, 1984). Adaptation is achieved through genetic changes over generations, which involves an evolutionary process, and through environment stimulation and experiences during an animal's lifetime, which involve ontogenetic processes (Price, 1984). Domestication is the first step of selection and has to be distinguished from taming, in that sense domestication means breeding (by choice of the reproducers and isolation from wild counterparts), care (shelter, food, protection against predators) and feeding of animals are more or less controlled by humans. Therefore, simply rearing animals in an adequate environment for a species (as for oysters or mussels) cannot be considered as domestication.

A domesticated animal is defined as an animal selectively bred in captivity and thereby modified from its ancestors, for use by humans who control the animal's breeding and food supply (Diamond, 1999). On the other hand, a domestic animal or one descended from a domestic population cannot revert to being a truly wild animal. Domesticated animals that return to nature to survive and breed are termed "feral". The distinction is a nice one, and intellectually useful, but not necessarily satisfying to a person who has had lambs killed by "wild dogs". The wild ancestor of the dog was the wolf, but the dog has

changed sufficiently in characters of bone, brain, and teeth that when it returns to nature it remains a dog for all of its wild behaviour.

Human has domesticated very few species. In the case of livestock, among 148 non-carnivorous mammal species weighing more than 45 kilograms, only 14 have been domesticated (Diamond, 1999). Thirteen of these species come from Europe or Asia and only one from America (the llama). The proportion is even lower in birds, with 10 of around 10,000 species being domesticated. Domestication of fish is beginning in a few species. There are more than 40 species of animals that have been domesticated or semi domesticated. Common species include cattle, sheep, goats, chickens, pigs, horses and buffalo, but many other domesticated species such as camels, donkeys, elephants, various poultry species, reindeer, rabbits, are important to different cultures and regions of the world (Rege & Gibson, 2003). The small number of domesticated species can possibly be explained by the characteristics required for domestication, including traits such as diet, reproduction, social relationships and behaviour towards human. Among these characteristics, the most important are a strong gregariousness (Diamond, 1999), feeding regimes that can be easily supplied by humans, which may explain why carnivores are scarce among domestic species, and precocious young (Diamond, 2002).

A modern human has never observed the natural processes of domestication by primitive people. Probably taming, and then domestication, occurred without people having been aware of what was happening. Certainly, gatherers and hunters - the people who first domesticated animals - could not have foreseen any uses for those animals other than those they knew already: for meat, bones and skins. Only later, after long experience and the intensification of a more sedentary life-style, and after the accumulation of random mutations and strong selections by human in domesticates, would secondary uses of animals - such as for milk, wool, motive power, war, sport, and prestige - realized (Reed, 1984).

Additionally, in the early history of domestication, all of the animals involved were social; humans, too, are social animals. Each social group learned to expand its tolerance to accept, in part, members of the other species as a part of a larger social group. Domestication could not have arisen otherwise in the beginning; the young of wild or half-wild mothers had handled and fed at a personal and individual level, so wildness would not develop. In each generation, those young that inherited genetic combinations for continuing wildness either escaped or were killed, so their genes did not persist in the population undergoing domestication.

2.1.2 Sheep Domestication

In the first place, such cultural change did not occur until after the evolution of anatomically modern (post-Neanderthal) humans and even then, not for almost 30000 years, so the emergence of people like us did not automatically result in domestication. The second main factor may have been the worldwide change in environment that accompanied and followed the end of the last glacial period. The earth became warmer, and the continental ice sheets began to melt back, extremely slowly at first, some 18000 YBP (Bruford *et al.*, 2003). Soon after this time, we find the first evidence of dogs, in south-western Asia at 14000 YBP. Three thousand years later, the ice sheets were in full retreat all over the world, so remarkably those geologists proclaim this time of c.l1500 YBP as the end of the Pleistocene and the beginning of the Holocene.

Sheep were domesticated entirely within the prehistoric period by primitive people living at the end of the Mesolithic (Middle Stone Age) period. The first animals to be domesticated after the dog were the goat and the sheep. Whether the goat or the sheep was domesticated first is not yet clear, because of the fragmentary nature of the skeletal remains, and the difficulty of distinguishing sheep and goat bones (Ryder, 1984).

2.1.3 Archaeological Signature in Sheep Domestication

Archaeology signature of initial domestication in sheep can be divided into two major categories: the first, those that reflect the evolutionary impact of domestication, and those that reflect human goals on managing animal population (Zeder *et al.*, 2006). Animal-oriented markers of initial domestication are those that signal the evolutionary divergence of domestic animals from wild ancestors and the response of managed animals to new, selective pressures introduced when human assume control over the breeding, movement, feeding, and protection from predators. These include a range of morphological changes in the form, size, proportions, and even the internal structure of bone (such as reduction of length of male horns, sexual dimorphism in horn size and body size reduction). Domestication also has had a distinct but somewhat less dramatic impact on the horns of sheep, consisting primarily of a reduction in the size of male horns and a tendency for hornlessness in domestic females.

Study for sheep domestication based on morphological changes in the form and size of the horns has unclear, consisting primarily of the presence of cranial fragments of

hornless females at early sites. The recovery of the skull of a hornless female sheep in basal levels of Ali Kosh, in western of Iran, for example, was used to argue for the presence of domestic sheep for the period of the earliest occupation of this site.

Horn size is also closely linked to the age of an animal, especially in male. Without knowing the age at sheath of the animal, it is difficult to say whether an apparent reduction in the length of breadth of horn sores in an archaeological assemblage is a reflection of horn size reduction resulting from domestication or simply a shift toward use of younger animals. Other markers are demographic factors and changing in body size reduction. For quarter of a century, body size reduction has been the primary marker of animal domestication in livestock species (Dobney & Larson, 2006).

2.1.4 The Wild Ancestor of Domestic Sheep

The Asiatic mouflon (*O. orientalis*), Urial (*O. vignei*) and Argali (*O. ammon*) occupy the extending from southwest to eastern Asia, and all are candidate ancestors of modern domestic sheep. Urial and Argali are now thought to be the least likely domestic ancestors according to studies of ovine mtDNA (Hiendleder *et al.*, 2002; Hiendleder *et al.*, 1998) and karyotype (Nadler *et al.*, 1973; Valdez *et al.*, 1978).

2.2 *Ovis* Taxonomy and Classification

2.2.1 Bovidae Family

The family Bovidae (Mammalia, Ruminantia) is diversified with 140 extent species classified into 45 genera (Grubb, 1993). Bovids are distributed in all continents where they occupied diverse ecological niches, but they never reached Antarctic, Australia, and South America. All bovids are clearly united by an unmistakable synapomorphy, for example, the possession of typical horns in males and sometimes in females, which are composed of a bone core covered by a permanent unforked keratinous sheath (Ropiquet & Hassanin, 2004). In the fossil record, the group emerged near 18.5 MYA (Vrba & Schaller, 2000). From the taxonomic point of view, the family Bovidae is one of the most problematic groups within mammals. The evolutionary relationships among most bovid species remain not fully resolved because of the complexity in their evolutionary mechanisms including temperature adaptation, feeding ecology, vegetation physiognomy and climatic fluctuations

(Kingdon, 1989), especially for species of the subfamily Caprinae, whose mountainous habitats have led to relatively poor fossil records (Simpson, 1945). Therefore, classification has been based primarily on morphology, behavior, ecology, chromosome number, and recently, molecular comparisons.

Within bovids, species of caprines are not united by an unambiguous morphological synapomorphy. The absence of diagnostic feature probably explains why the composition of this group is considerably variable in the literature. The tribe Caprini sensu lato (subfamily Antilopinae) included the following 11 genera: (1) *Ammotragus* (aoudad), (2) *Budorcas* (takins), (3) *Capra* (goats, ibexes, markhor and turs), (4) *Hemitragus* (tahrs), (5) *Naemorhedus* (gorals and serows), (6) *Oreamnos* (Rocky Mountain goat), (7) *Ovibos* (muskox), (8) *Ovis* (sheep, argali and mouflons), (9) *Pantholops* (chiru), (10) *Pseudois* (bharals) and (11) *Rupicapra* (chamois and isards) (Ropiquet & Hassanin, 2004; Ropiquet & Hassanin, 2005).

2.2.2 *Ovis* Taxonomy and Classification Problems

The genus *Ovis*, which includes all true sheep, constitutes one of the more complex mammalian genera relative to its evolution and systematic. Based on morphological data, numerous wild sheep classifications and revisions have been proposed during the last two centuries (Hiendleder *et al.*, 2002). A basic difference lies in the number of species recognized. Tsalkin (1951) proposed two species (*O. ammon*, *O. nivicola*/*canadensis*). However, Haltenorth (1963) even proposed a single polymorphic one (*O. ammon*). Valdez, (1982) recognized five species of sheep (*O. ammon*, *O. dalli*, *O. canadensis*, *O. nivicola*, and *O. orientalis*). Wilson and Reeder (1993) proposed six species for sheep (*O. ammon*, *O. dalli*, *O. canadensis*, *O. nivicola*, and *O. aries*). The classification based on chromosome number and geographical distribution by Nadler (1973) recognized seven species. These are European mouflon (*O. musimon* 2n = 54), Asiatic mouflon (*O. orientalis* 2n=54), urial (*O. vignei*, 2n = 58), argali (*O. ammon*, 2n = 56) and, Dall sheep (*O. dalli*, 2n = 54), bighorn (*O. canadensis*, 2n = 54) and snow sheep (*O. nivicola*). However, Siberian snow sheep were later shown to have a karyotype of 2n = 52 (Korobytsina *et al.*, 1974; Nadler *et al.*, 1974). According to natural habitat range overlapping, the different species of the genus *Ovis* can hybridize and produce fertile offspring (Nadler *et al.*, 1971; Valdez *et al.*, 1978). For instance, the mouflon/urial hybrid zone in northern and south-eastern Iran displays individuals with intermediate chromosome

numbers between 54 and 58. These data have been interpreted as a support for a single 'moufloniform' species (*O. orientalis*), comprising mouflon and urial populations (Valdez, 1982; Valdez *et al.*, 1978). The International Union for the Conservation of Nature and Natural Resources (IUCN) has used this classification of only three species (*O. orientalis*, *O. ammon* and *O. nivicola*) of Eurasian wild sheep (Shackleton & Lovari, 1997).

2.2.3 Illustrations of the Species and of the Geographic Distribution

The wild *Ovis* are composed of seven groups based on different morphologies, chromosome number and geographic distributions. Thus, we chose to use the classification that recognized seven species (Nadler *et al.*, 1973).

The argali (*Ovis ammon*, Linnaeus 1758) is the largest wild sheep, weighing in between 60 and 200 kilograms and shoulder heights upto 120 centimetres. Horns of argali are longest and heaviest of the wild sheep (Figure 1.1). Argali horns have two full circles of spiral, with tops always directed sideways; this pattern is distinct from that of other *Ovis* species (Fedosenko & Blank, 2005). Argali range from the Russian and Mongolian Altai and the Gobi desert to Inner Mongolia, China, Trans-Alai and Alai ranges, eastern Pamir, the Tibetan Plateau, as well as Himalayas in Ladakh, Nepal, Sikkim, and Bhutan.

Figure 2-1 Argali sheep (*Ovis ammon*)

The Asiatic and European mouflon (*O. orientalis*, Pallas 1811) are found in the west of Asia and the west of Europe. Rams have a distinct white rump patch and a black or white ruff on the front of the neck in winter coat. Horns are comparatively slender (Figure 1.2a). Ewes may grow short, thin horns in some subspecies, but are commonly hornless in

others. European mouflon (*O. orientalis musimon*, Pallas 1811) was until recently only found wild in the mountains of Corsica and Sardinia but it has been successfully introduced as a wild animal to many European countries now (Figure 1.2b). It used to be thought by biologists and sportsmen that the European mouflon was a truly wild species, a relic of the European wild sheep of the Pleistocene that lived only in the refuge area of Mediterranean islands. The lack of fossil evidence for sheep on the islands as well as in Europe weighs against this theory (Poplin, 1979; Vigne, 1988).

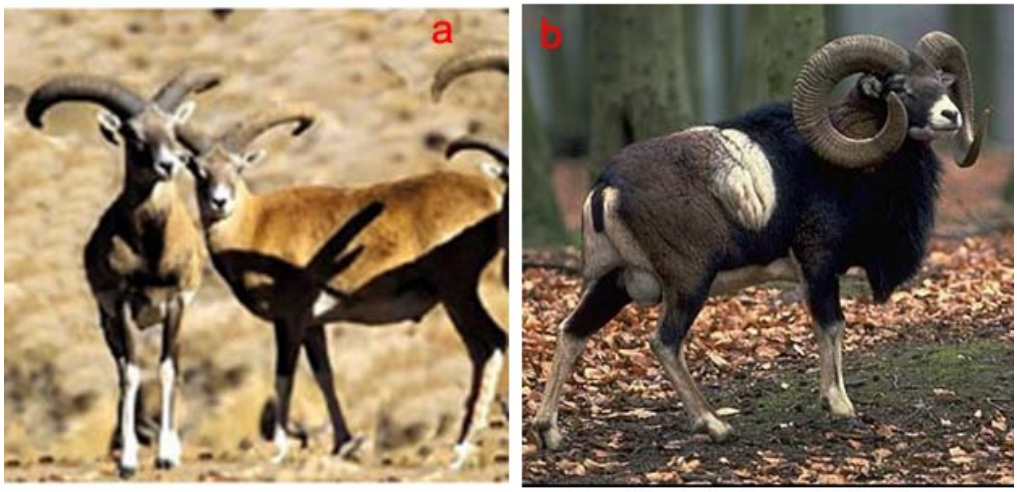


Figure 2-2 Asiatic mouflon *O. orientalis* Bozdağ (Konya) Turkey (a) and European mouflon *O. orientalis musimon* Corse Island, France (b)

It is now accepted that the European mouflon, rather than being a relic of a wild species, is a relic of the first domestic sheep that arrived to Europe by the early Neolithic farmers about around the seventh millennium BC (Poplin, 1979).

The Urial (*O. vignei*, Blyth 1841) has brown colour with a lighter coat in summer than in winter. They have a distinct white rump patch below the base of the tail and along the back of the hindquarters. Males have a black neck ruff, which is restricted to the front of the neck and brisket. The urial sheep are widely distributed in Asia Minor. They can be seen southwest Kazakhstan through Turkmenistan, Uzbekistan, Tajikistan, Afghanistan, Pakistan, Iran and Kashmir region of India (Figure 1.3).

Figure 2-3 Urial *O. vignei*. Golestan National Park, Iran

The Bighorn (*O. canadensis*, Shaw 1804) sheep's muscular bodied animal is covered with a brown coat, the belly, rump, back of legs, muzzle and eye patch are white. The most distinct feature of the mature male Bighorn is a set of massive horns, which spiral backwards from the top of the head (Figure 1.4). The hooves are hard on the outside and soft on the inside making it an excellent climber and jumper. The bighorn is found in the Rocky Mountains from Canada to Colorado and a desert subspecies from Nevada and California to west Texas and south into Mexico.

Figure 2-4 Bighorn (*O. canadensis*)

Dall sheep or tinhorn (*O. dalli*, Nelson 1884) is a wild sheep of the mountainous regions of western Canada and United States, The Dall sheep is smaller than the bighorn, has more slender and gracefully curved horns, and is white in colour. On a dark background, Dall sheep appear to be pure white, but in the snow, they are seen to be slightly yellowish.

Figure 2-5 Dall sheep (*O. dalli*)

Snow sheep (*O. nivicola*, Eschscholtz 1829) inhabit the most northern range of the Eurasian wild sheep, which comprises an expanse of mountain ranges in northern Russia that is larger than the lower continental United States (Bunch *et al.*, 2006). A small patch of light hair on the buttocks accents the greyish brown coat. The woolly winter coat is a light, milky coffee colour. The fronts of the legs are dark chocolate brown, while the rear edges may have whitish markings. The ears are small and dark grey in colour. The horns, found in both sexes, are considerably lighter than those of the related Bighorn sheep.

Figure 2-6 Snow sheep (*O. nivicola*)

The domestic sheep (*Ovis aries*) is the most common species of the *Ovis* genus. Today, over 2370 sheep breeds are recognized worldwide. Europe supports a greater number of breeds than any other continent (FAO, 2004). They are different in many ways including size, the length and texture of their wool, the form and size of their horns and the length of their tail (Figure 1.7). These breeds are generally sub-classable as wool class, hair class and sheep meat variety breeds. Dual-purpose breeds are bred for both wool and meat.

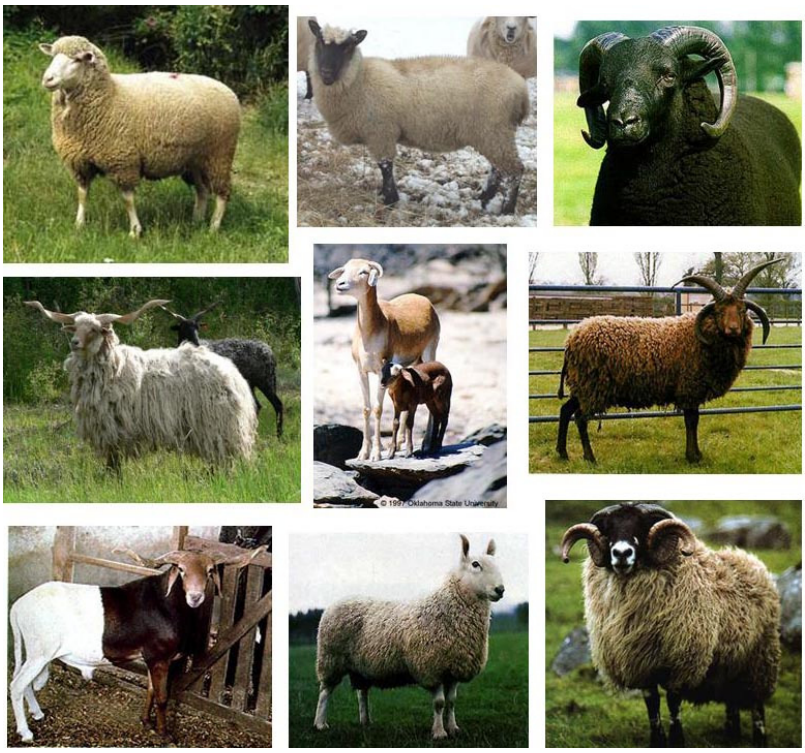


Figure 2-7 Domestic sheep (*O. aries*)

2.2.4 General Biology

The hoofed herbivores walk on 'tip-toe' as a specialization for speed and cloven hoofs are well adapted to walking on soft ground as well as to climbing the stony slopes of the recent natural mountain home of the sheep and goats (family Bovidae, subfamily Caprinae). Sheep can be distinguished from cattle by their narrow, hairy and cleft upper lip, which allows them to graze closer and more selectively than cattle. They also have only one pair of nipples compared with two pairs in cattle.

The cud chewing habit is probably the single most important factor contributing towards the evolutionary success of the ruminant group. Domestic sheep must nevertheless

spend 9-11 hours of each day grazing, and 8-10 hours, mostly at night, ruminating. Cud chewing is associated with a four-chambered stomach and a specialized digestive system involving the fermentation of cellulose by micro-organisms in the rumen, which allows the animal to derive nutriment from fibrous material.

Wild sheep breed in November and December and have a gestation period of about 5 months. They have adapted to high latitudes and cold climates by either delaying the breeding season (which is controlled by day length) or by extending the gestation length (Geist, 1971). There appears to be no consistent association of breeding season with latitude in domestic sheep, and one wonders whether the onset has been hastened by selective breeding. The main season lasts from the beginning of September until the end of November in the northern hemisphere, and ewes quickly adjust to a transfer between hemispheres.

The Merino and most tropical breeds can breed all the year round, and this has been attributed to their evolution in latitudes with little seasonal change. Among temperate breeds, the Dorset Horn is notable for its long breeding season, which starts as early as June. The Finnish Landrace is in season from early October until mid-May, which could be regarded as an adaptation to high latitudes.

Rams can produce sperm throughout the year, although there is a tendency towards quiescence during the summer months in breeds with ewes having a restricted season. In some breeds, both sexes can mate as young as 6 months.

Numerical attribution of acrocentric chromosome equivalents based on the fundamental karyotype of *Ovis* that gave rise to the biarmed chromosomes are 1 and 3 for the largest biarmed chromosomes. The G-banding patterns of this largest pair of chromosomes were identical in all wild and domestic sheep. The urial (*O. vignei*, 2n = 58) has only one pair of biarmed chromosome. All other species of genus *Ovis* with 2n = 56, 54, and 52 have the second biarmed pair. The latter arised from fusion of acrocentrics 2 and 8. The third resulted from the fusion of acrocentrics 5 and 11, resulting in the 2n = 54 karyotype, and is maintained in the domestic sheep (*O. aries*), mouflons (*O. orientalis*), all North American wild sheep (*O. canadensis* and *O. dalli*) and the snow sheep with a 2n = 52. The most recently evolved *Ovis* karyotype arose from acrocentrics 9 and 19, and is exclusive to the snow sheep (*O. nivicola*) (Bunch, 1978; Bunch *et al.*, 1976; Bunch & Nadler, 1980; Bunch *et al.*, 1998; Bunch *et al.*, 2000; Bunch *et al.*, 2006; Mensher *et al.*, 1989; Nadler & Bunch, 1977). The 2n = 52 karyotype of *O. nivicola* most likely occurred after disruption of the Bering land bridge 12,000 years ago (Korobytsina *et al.*, 1974).

2.2.5 Mitochondrial (mt) DNA and His Role in Genetic Research

To help understand the origins of domestication of a livestock species, an ideal molecular marker should have several characteristics. First, it should be sufficiently evolutionarily conserved to allow the identification of the wild taxon or population from which the species descends. Second, the marker should be variable and structured enough across the geographical range of the species so that the approximate locality of domestication can be identified. Third, the marker should evolve at a rapid but constant rate; this feature allows the origin of a particular polymorphism to be dated. This combination of characteristics is difficult to find, but fortunately, in animal evolutionary studies, there is such a marker: mtDNA. The average rate of synonymous substitutions in mtDNA is about 20 times higher than in nuclear DNA (Pesole *et al.*, 1999). At present, mtDNA is by far the most widely used molecular tool in domestication studies (Bruford *et al.*, 2003). The mtDNA can also tell us about the recent demographic processes affecting a population, for example, whether a population has undergone a recent demographic expansion, or has a more complex history. Mammalian mtDNA is also almost exclusively maternally inherited, is effectively haploid and does not undergo recombination. These characteristics mean that each individual has a single haplotype and that phylogenetic analyses are relatively straightforward to interpret.

The mtDNA is routinely used to produce phylogenetic trees at several taxonomic levels, from within species to among orders of mammals. In livestock, it has been used to describe variation in putative wild ancestor populations and modern domestic populations.

Structure and gene organization of mtDNA are conserved in mammals. The mitochondrion is an organelle in the cell cytoplasm found outside the cell nucleus. It is the only animal organelle with its own DNA. In mammals, mtDNA is transmitted to the progeny only from mother (Giles *et al.*, 1980; Hayashi *et al.*, 1978; Hutchison *et al.*, 1974). The possibility for rare paternal inheritance and recombination among mitochondrial lineages has been suggested, but this remains controversial (Gyllensten *et al.*, 1991; Piganeau & Eyre-Walker, 2004; Piganeau *et al.*, 2004). Animal mitochondrial DNA represents a closed circular molecule about 16600 base pairs consisting of 2 ribosomal RNA (rRNA) genes, 22 tRNA genes, 13 protein-encoding genes, and a non-coding control region associated with the origin of heavy strand replication (Brown, 1985). Relative to the nuclear genome, mtDNA evolves at a faster rate (Wilson *et al.*, 1985), with different

regions of the genome displaying a wide array of rates, thus making the molecule ideal for within- and between-species comparisons.

Studies of intraspecific phylogeographic, which reveal patterns of variation resulting from either historical or recent barriers to gene flow between populations, were initiated using mtDNA (Avise *et al.*, 1998). These patterns were used to identify highly divergent geographic regions that showed concordant patterns across aquatic and terrestrial organisms. The identification of such regions of endemism provides an excellent tool for the management and conservation of genetically distinct units (Proudfoot *et al.*, 2006).

Two distinct haplogroups were recorded in the first surveys of sheep mtDNA variation (Hiendleder *et al.*, 2002; Hiendleder *et al.*, 1998; Wood & Phua, 1996; Zardoya *et al.*, 1995) and third distinct haplogroup was reported (Guo *et al.*, 2005; Pedrosa *et al.*, 2005). Recently, two new haplogroups were reported (Meadows *et al.*, 2007; Tapio *et al.*, 2006).

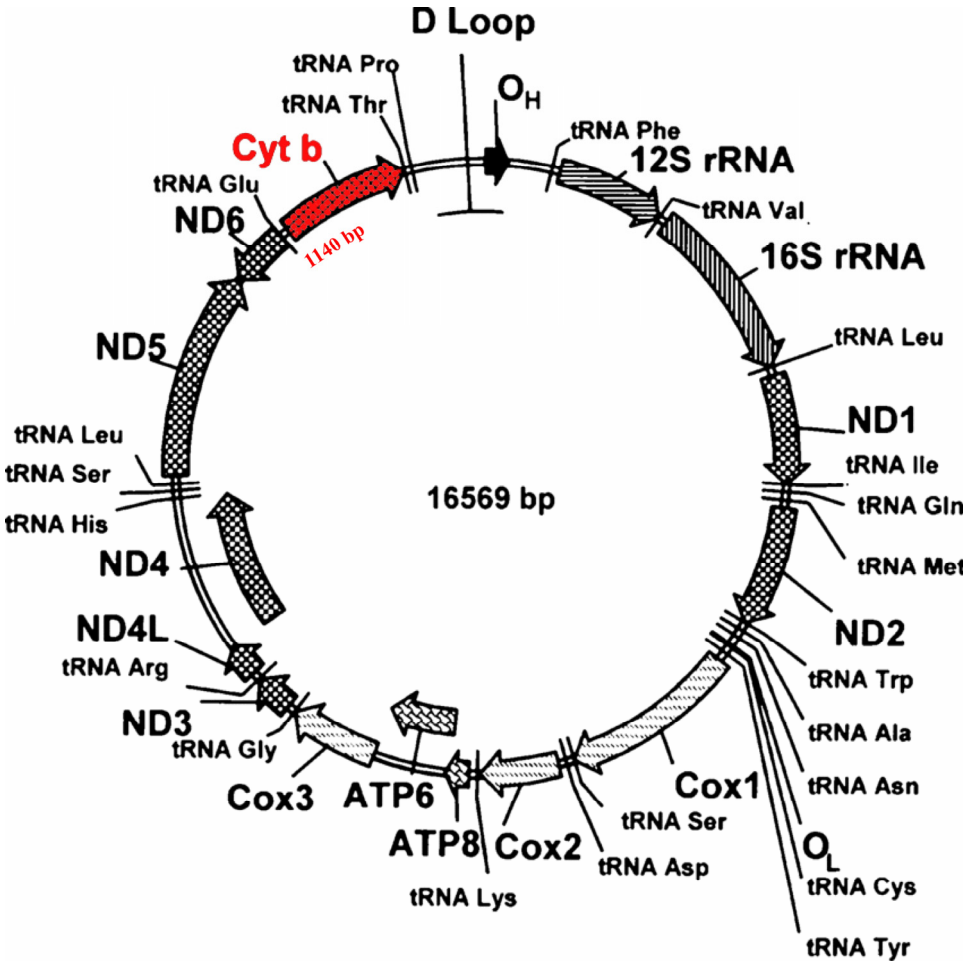
2.2.6 Cytochrome *b*

This gene has proven to be especially important in livestock studies, because its tempo and mode of evolution is well understood and is thought to be relatively constant and similar among large-bodied terrestrial mammals. It is the only cytochrome coded by mtDNA.

The Cyt*b* gene is the most widely used gene for phylogenetic work for several reasons. Although it evolves slowly in terms of non-synonymous substitutions, the rate of evolution in silent positions is relatively fast (Irwin *et al.*, 1991). The wide use of cytochrome *b* has created a status as a universal metric, in the sense that studies can be easily compared. Cyt*b* is thought to be variable enough for population level questions, and conserved enough for clarifying deeper phylogenetic relationships. However, the cytochrome *b* gene is under strong evolutionary constraints because some parts of the gene are more conserved than others due to functional restrictions (Meyer, 1994). Most of the variable positions seem to be located within the coding regions for transmembrane domains or for the amino- and carboxy-terminal ends (Irwin *et al.*, 1991).

The Cyt*b* gene has been used in numerous studies of phylogenetic relationships within mammals, and it is the gene for which the most sequence information from different mammalian species is available (Castresana, 2001; Hassanin & Douzery, 1999; Hassanin *et al.*, 1998; Irwin *et al.*, 1991; Johns & Avise, 1998; Meyer, 1994). The sequence

variability of Cyt*b* makes it most useful for the comparison of species in the same genus or the same family. The results obtained in many of the phylogenetic studies in which this gene has been used led to the proposition of new classification schemes that better reflected the phylogenetic relationships among the species studied (Arnason *et al.*, 1995; Hassanin & Ropiquet, 2004; Lara *et al.*, 1996; Matthee & Robinson, 1999).



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2.3 Conservation Genetics

The biodiversity of the planet depleted rapidly as a direct and indirect consequence of human actions. An unknown but large number of species are already extinct, while many others have reduced population size that put them at risk (Frankham, 2003). Approximately 25% of mammals, 11% of birds, 20% of reptiles, and 34% of major plant taxa are threatened with extinction over next few decades (IUCN, 2001). Many species now require kindly human intervention to improve their management and ensure their survival. The most important factors contributing to extinction are habitat loss, introduced species, over exploitation and pollution. Conservation genetics deals with the genetic factors that affect extinction risk and genetic management regimes required to minimize these risks. There are many major genetic issues in conservation biology such as inbreeding depression, loss of genetic diversity, fragmentation of population and reduction in gene flow. Even if the original cause of population decline is removed, problems related to small population size will persist.

Identification of management units is necessary so that management and monitoring programs can be efficiently targeted toward distinct or independent populations. Biologists and ecosystem managers must be able to identify populations and geographic boundaries between populations in order to effectively plan harvesting quotas (for example, to avoid over harvesting) or to devise translocations and reintroductions of individuals (for example, to avoid mixing of adaptively differentiated populations). In addition, it is sometimes necessary to prioritize which population units (or taxa) to conserve because limited financial resources preclude conservation of all units (Allendorf & Luikart, 2007).

The identification of appropriate taxonomic and population units for protection and management is essential for the conservation of biodiversity. For species identification and classification, genetic principles and methods are relatively well developed; nonetheless, species identification can be controversial. Within species, the identification and protection of genetically distinct local populations should be a major focus in conservation because the conservation of many distinct populations helps maximize evolutionary potential and minimize extinction risks (Hughes *et al.*, 1997; Luck *et al.*, 2003). Furthermore, the local population is often considered the functional unit in ecosystems.

2.3.1 Animal Genetic Resources

The term "animal genetic resources" is used to include all animal species, domestic breeds and their wild relatives that are of economic, scientific and cultural interest to humankind in terms of food and agricultural production for the present or in the future.

Livestock genetic resources underlie the productivity of local agricultural systems. They also supply a resource of genetic variation that can be exploited to provide continued improvements in adaptation and productivity. Thus genetic erosion within livestock species, including their wild ancestors, is of particular concern because of its implications for the sustainability of locally adapted agricultural practices and the consequent impact on food supply and security (Rege & Gibson, 2003).

The selected species accompanied human populations across the earth, evolving through a combination of natural and human selection to adapt. The current enormous genetic diversity of domestic animal genetic resources represented in today's breeds and strains is the result of 12000 years process. Once lost, such diversity will be all but impossible to recreate. Existing animal genetic resources thus represent a massive past investment that if managed appropriately can provide insurance against an unknowable global future.

2.3.2 Wild and Domestic

Biodiversity conservation becomes associated mainly with issues related to wild plants and animals. Although much less discussed, the loss of farm animal genetic resources may well be much more serious than in crops because the gene pool is smaller and very few wild relatives remain (Taberlet *et al.*, 2007). The value of both traditional farmers' varieties and wild relatives of cultivated plants in crop improvement and agricultural development cannot be overemphasized (Esquinas-Alcazar, 2005). The fact that 32% of livestock breeds worldwide are at risk of becoming extinct and that the rate of extinction continue to accelerate (FAO, 2004) is thus a serious cause of concern. Livestock supply some 30% of total human requirements for food and agriculture, while 70% of the world's rural poor inhabitants depend on livestock as an important component of their livelihoods. Animals are of different characteristics and hence outputs suit differing local community needs. The loss of crop and livestock diversity seriously reduces our potential

to alleviate poverty, improve food security, and promote sustainable agriculture (Esquinas-Alcazar, 2005).

In domestic species, conservation typically operates at the level of breeds, not at the level of species. The most important arguments for conservation of domestic breeds concern maintaining or increasing food production to keep pace with global environmental changes, opportunities to meet future market demands, possibilities to offer livelihoods for people, both locally and globally, together with cultural-historic and scientific reasons (Garner *et al.*, 2005). The practical conservation goals center on maintaining the greatest possible genetic variation in the species, maintaining particular populations and adaptations. In addition, the practical conservation goals focus on ensuring survival of the populations chosen for conservation without unnecessary loss of within-population variation and avoidance of inbreeding. In the context of wild mammalian species, substantial losses of genetic diversity occur at the population or subpopulation level before the species becomes endangered. Studies in domestic species reveal how, for instance, management and population admixture can influence diversity and which kinds of populations are important to maintain variation in the species.

2.3.3 What to Conserve

The discipline of conservation genetics focuses on preserving genetic diversity in populations subjected to fragmentation, reduction in census size and other perturbations (Hedrick & Miller, 1992). Often, ecosystem managers are interested in estimating how these factors interact to determine population viability. Genetic techniques can also be valuable in evaluating management strategies for populations such as the effects of introducing outside individuals into inbred populations (Madsen *et al.*, 1999; Westemeier *et al.*, 1998). If population restoration is attempted, genetic analyses can help determine which populations should serve as source stocks, optimal scenarios for maintaining population genetic diversity, and which should be maintained as unique genetic units (Maudet *et al.*, 2002b).

According to the World Watch List, out of the around 6300 breeds registered by FAO, 1350 are threatened by extinction or are already extinct (FAO, 2004). Threats to genetic diversity include wars, pests and diseases, global warming, urbanization, intensification of agriculture and global marketing of exotic breeding material. However, by far the greatest cause of genetic erosion is failure to appreciate the value of locally

adapted breeds. In many countries, farmers rely on a very limited number of modern breeds that are most suited for intensive agriculture systems. Many developing countries still consider breeds from industrialized countries to be more productive, although they have difficulties in coping with the often-harsh environment.

Intensive production and increased commercial demands, particularly since the end of the Second World War, have significantly contributed to the threats facing European sheep breeds. Artificial insemination and improved transportation have reduced the number of breeding rams, leading to a reduction in the effective population size of many breeds. Also, production has focused on only a few breeds to the detriment of rare or minority breeds, which are likely to be important genetic resources because of their local adaptation, disease resistance, high fertility and unique product qualities (Mendelsohn, 2003). Minority breeds have been lost by introgression from large commercial populations to*o*.

Information on both within and among breed diversity is important. The former provides information for management at the breed level. The latter helps to identify divergent breeds that may harbors distinct genotypes and are, therefore, worthy of conservation efforts even if their within breed diversity is relatively high.

The implications of the many recent molecular genetic studies for different domestic species are clearly different in each case. However, the relevance of this information and how it might be incorporated in management plans for endangered livestock has some general implications.

First, although the wild progenitor species are extinct for some species (such as cattle and horses), the identification of ancestral populations for other livestock could be very important for two reasons. It is probable that some are endangered and such information might give extra impetus for their conservation. Moreover, ancestral populations (and closely related species) might be a source of alleles of economic value that have been lost by chance during domestication.

Second, the characterization of genetic diversity within and between breeds, and the identification of the geographical component of this variation, allows region specific conservation measures to be put in place. For some domestic species in Eurasia, the most eastern breeds or those nearest the putative centers of domestication have repeatedly been shown to contain greater genetic diversity than breeds located further away from these points. Management strategies and global priorities for the maintenance of genetic

diversity must not ignore these data: these higher diversity breeds should receive a concomitant higher priority for conservation.

From a purely anthropocentric perspective, another major value of wild *Ovis* is that it was the ancestor of one of the most important species of domestic livestock – domestic sheep (*Ovis aries*). In attendance, populations of wild sheep species represent a potential source of new genetic material that can be used to improve or adapt current domestic breeds to less productive conditions (Shackleton, 1997).

2.4 Objectives

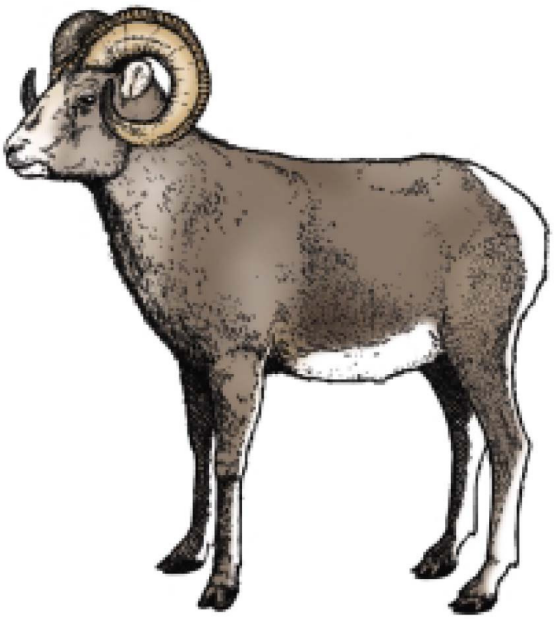
A definition of domestication, *Ovis* taxonomy and classification, domestication of sheep and the importance of conservation genetics were documented in this chapter. As it is stated through the chapter, the wild species of *Ovis* genus have intrinsic value as part of megafauna of a wild range of distinct ecosystems. Throughout most of their distribution, wild *Ovis* has great importance for both consumptive (such as hunting for food and sport) and non-consumptive (such as ecotourism) uses.

However, the classification and taxonomy of *Ovis* species are confused. Particularly, mouflon (*O. orientalis*) and urial sheep (*O. vignei*) require special mention. They are classified as a single species (*O. orientalis*) or as separate species. Part of the problem revolves around the total chromosome numbers for speciation (Valdez *et al.*, 1978). In addition, morphological factors can be varied in the species hybrid populations. Chapter 3 will discuss how mitochondrial phylogeny can explain the classification of *Ovis* genus, which is the main aim of the chapter.

The questions of how, when, where and why people first domesticated the animals are central to an understanding of the history of humanity (Harris, 1996). To answer to these questions, a part of mitochondrial and nuclear DNA of wild and domestic sheep were compared. The comparison results itself was compared with archaeological data. The results are documented in Chapter 4. Both chapters two and chapter three are pressed on manuscripts of papers.

Since the beginning of the domestication, the farmers have started to manage and select the breeds. However, in the last two centuries, the rate of the selection was increased. With the reason of inbreeding within the breeds, the domestic animals are currently losing genetic diversity through many mechanisms (Taberlet *et al.*, 2007). There are many questions such as what the optimal management guidelines for a sustainable use

of genetic resources in cattle, sheep and goats are. Are cattle, sheep, and goats endangered species? These questions are answered in Chapter 5. The chapter begins with a synthesis from the data of the literature for the identification and filiation genetics making it possible to apprehend the research topic. This chapter is accepted as a paper in Molecular Ecology in 2007.



Chapter 3

Evolution and Taxonomy of the Wild Species of the *Ovis* Genus

3. Evolution and Taxonomy of the Wild Species of the *Ovis* Genus (Mammalia, Artiodactyla, Bovidae) Inferred from a Mitochondrial Phylogeny

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3.1 Abstract

The systematic of the *Ovis* genus is controversial and several classifications have been proposed. Seven main groups of wild sheep are distinguished on the basis of different karyotype, morphologies and geographic distributions. New insights for the systematic and evolution of the wild sheep are provided by cytochrome *b* phylogenies inferred from Bayesian, maximum likelihood, and neighbour joining methods. First, a phylogeny of the Caprinae family based on 28 species including 2 samples from each *Ovis* taxon confirmed the monophyly of the *Ovis* genus. Then 235 samples covering the whole geographic distribution area and representative of most of the subspecies were used for the phylogeny of the wild sheep. In this phylogeny urial and mouflon, which are either considered as a single species (*Ovis orientalis*) or as two separate species (*O. orientalis* and *O. vignei*), form two monophyletic groups strongly supported by high bootstrap values. Hybrids between *O. vignei* and *O. orientalis* appear in one or the other group, independently from their geographic origin within the hybrid zone. The European mouflon *Ovis musimon* is clearly in the *O. orientalis* clade. The other species, *O. dalli*, *O. Canadensis*, *O. nivicola* and *O. ammon* are monophyletic. As a whole, the results support the hypothesis of an Asiatic origin of the genus *Ovis*, followed by migration to North America through North-Eastern Asia and the Bering Strait and a diversification of the genus in Eurasia between 3 and 5 MYA.

Keywords: Molecular Phylogeny; Taxonomy; cytochrome *b*; *Ovis*; Urial; Mouflon; Caprinae;

3.2 Introduction

The genus *Ovis* is one of the more complex mammalian genera with regard to its evolution and systematic. Based on morphological criteria and geographic distribution, several classifications and revisions have been proposed during the last two centuries (summarized in Hiendleder *et al.,* 2002, see Table 3-1). Haltenorth, (1963) proposed that all wild sheep were polymorphic populations of a single species (Tsalkin, 1951; Valdez, 1982; Wilson & Reeder, 1993). Up to seven species have been recognized (Nadler *et al.*, 1973). They differ in morphological traits such as body size, horn morphology, colour and pattern of the coat (Fedosenko & Blank, 2005), in chromosome number and in their geographic distribution (Figire 3-1). The European mouflon (*O. musimon*, 2n = 54) and the Asiatic mouflon (*O. orientalis/gmelini*, 2n = 54) are found in the west of Asia and Europe, the Argali (*O. ammon*, 2n = 56) lives in mountainous areas in central Asia, and the Urial (*O. vignei*, 2n = 58) is widely distributed in Asia Minor. The Dall sheep or Tinhorn (*O. dalli*, 2n = 54) lives in the mountainous regions of western Canada and United States, the Bighorn (*O. canadensis*, 2n = 54) is found in the Rocky Mountains from Canada to Colorado and south to Mexico, and the snow sheep (*O. nivicola,* 2n = 52) is mainly found in the North East of Asia. The situation is even more complex given that different *Ovis* taxa with overlapping distributions hybridize and produce fertile offspring considered as subspecies (Nadler *et al.*, 1971; Valdez *et al.*, 1978). For example, there is a mouflon/Urial hybrid zone with individuals displaying intermediate chromosome numbers between 55 and 57 in northern and south-eastern Iran. This data supports the existence of a single 'moufloniform' species (*O. orientalis*) composed of mouflon, Urial and hybrid populations (Valdez, 1982; Valdez *et al.*, 1978). The current reference classification adopted by the International Union for the Conservation of Nature and Natural Resources (IUCN) is based on the Valdez's classification (Shackleton & Lovari, 1997), even if the status of *O. orientalis* as a unique species remains questionable. For clarity, we will follow the Nadler (1973), classification because it distinguishes the greatest number of taxonomic entities.

Molecular studies could help in understanding the evolution and taxonomy of the wild *Ovis,* but only partial information is available. Molecular phylogenies show that the *Ovis* genus is monophyletic (Hernandez Fernandez & Vrba, 2005; Ropiquet & Hassanin, 2004; Ropiquet & Hassanin, 2005) and diverged from the other Caprinae about 7 MYA (Hernandez Fernandez & Vrba, 2005) probably in Asia according to palaeontologists

(Vrba & Schaller, 2000). However, these studies did not consider all wild species of the *Ovis* genus. Moreover, the phylogenetic relationships within the *Ovis* genus, have only been studied between *O. nivicola* and its two close relatives *O. ammon* and *O. dalli* (Bunch *et al.*, 2006). Other molecular studies have dealt with subspecies of *O. canadensis* (Boyce *et al.*, 1999; Ramey, 1995) and *O. ammon* (Tserenbataa *et al.*, 2004; Wu *et al.*, 2003).

The lack of global molecular studies and the absence of concordance between available data call for a molecular phylogeny based on a large sample that represents the diversity of *Ovis* taxa. This study provides a cytochrome *b (*Cyt*b*) phylogeny of the wild *Ovis* species in order to infer their evolutionary history and to check the species or subspecies status of the taxa defined on morphological and karyotypic criteria.

3.3 Materials and Methods

3.3.1 Taxon Sampling and DNA Extraction

Samples from 235 *Ovis* were collected from 37 regions in Europe, Asia, USA and Canada (Table 3-2), thanks to several collaborations. Most of the samples were obtained using a non-invasive method. Fresh faeces where collected in the field, after having observed the sheep from a distance to ensure its species identification. This avoids capturing the animals and thus reduces the risk of injuries and of disturbing the social group (Taberlet *et al.*, 1999). Another advantage of using faeces is that CITES permission is not needed for species listed under annex 1 and 2 of the IUCN red list (IUCN, 2006). For each individual two samples were collected and preserved using two methods (silica gel and ethanol 96%). Some other samples consisted of skin and muscles obtained from winter hunter kills and do not concern species under CITES regulation. Because of a possible hybridization in captivity, no samples from zoos were considered in this study. The collected samples represented six species *O. vignei, O. gmelini, O. musimon, O. ammon, O. dalli, and O. canadensis* (according Nadler classification). The data set was completed with 18 Cyt*b* sequences of *O. ammon*, *O. orientalis*, *O. dalli, O. musimon* and *O. nivicola* obtained from Genebank (Table 3-3).

Table 3-1. The different classifications of the genus *Ovis*

Groups	Tsalkin [1]	Haltenorth [2]	Nadler *et al.* [3]	Valdez [4] Wilson & Reeder [5] Shackleton & Lovari [6]	Festa-Bianchet [7]
Dall Sheep	*O. canadensis*/*O. nivicola*	*O. ammon*`	*O. dalli*	*O. dalli*	*O. dalli*
Bighorn	*O. canadensis*/*O. nivicola*	*O. ammon*	*O. canadensis*	*O. canadensis*	*O. canadensis*
Snow Sheep	*O. canadensis*/*O. nivicola*	*O. ammon*	*O. nivicola*	*O. nivicola*	*O. nivicola*
Argali	*O. ammon*	*O. ammon*	*O. ammon*	*O. ammon*	*O. ammon*
Asiatic mouflon	*O. ammon*	*O. ammon*	*O. gmelini*	*O. orientalis*	*O. orientalis*
Urial	*O. ammon*	*O. ammon*	*O. vignei*	*O. orientalis*	*O. vignei*
European mouflon	*O. ammon*	*O. ammon*	*O. musimon*	*O. orientalis musimon*	*O. orientalis musimon*

[1] (Tsalkin, 1951), [2] (Haltenorth, 1963), [3] (Nadler *et al.*, 1973), [4] (Valdez, 1982), [5] (Wilson & Reeder, 1993), [6] (Shackleton & Lovari, 1997) and [7] (Festa-Bianchet, 2000).

The whole genomic DNA was extracted from fecal samples after 20 minutes in washing buffer (Tris-HCl 0.1 M, EDTA 0.1 M, NaCl 0.01 M, N-lauroyl sarcosine 1%, pH 7.5-8.0), using DNAeasy extraction blood kit (Qiagen) following the manufacture's protocol for animal blood except for the incubation with protease (2 hours at 56° C with 55 µl of protease). For tissue samples, total DNA was extracted using the tissue extraction kit QIAamp Animal Tissue kit (Qiagen) following the manufacture's instructions.

3.3.2 PCR Amplification and Sequencing

We sequenced the Cyt*b* gene that is useful for inferring Bovidae phylogenies (Groves & Shields, 1996; Hassanin & Douzery, 1999; Hsieh *et al.*, 2003; Janecek *et al.*, 1996; Pedrosa *et al.*, 2005; Pidancier *et al.*, 2006; Rebholz & Harley, 1999). The total mitochondrial Cyt*b* was amplified with two pairs of primers (Pedrosa *et al.*, 2005): CYTB_F (5'-CCCCACAAAACCTATCACAAA-3') and CYTB_IN_R (5'-CCTGTTTCGTGGAGGAAGAG-3') for the first part, and CYTB_IN_F (5'-ACCTCCTTTCAGCAATTCCA-3') and CYTB_R (5'-AGGGAGGTTGGTTGTTCTCC-3') for the second one. The PCR reactions were performed in a final volume of 25 µl containing 2 µl of DNA, 1 µM of each primer, 1x PCR buffer, 200 µM of each dNTP, 1.5 mM $MgCl_2$, and 1 unit of AmpliTag Gold polymerase (Applied Biosystems). PCR was performed according to the following protocol: initial denaturation, 95°C, 10 min; then for 35-40 cycles, denaturation, 95°C, 30 s; annealing, 55°C or 60°C (for CYTB_F/ CYTB_IN_R and CYTB_IN_F/CYTB_R, respectively), 30 s; extension, 72°C, 1 min; a final extension, 7 min, 72°C. PCR products were purified using the Qiaquick kit (Qiagen) following the manufacturer's instructions. Purified PCR products were used as the template in 20 µl BigDye Terminator Cycle Sequencing kit version 3.1 (Applied Biosystems) and analyzed on an ABI Prism 3100 automated sequencer (Applied Biosystems). SeqScape 2.5 (Applied Biosystems) was used to reconcile chromatograms of complementary fragments and to align sequences across taxa. As Cyt*b* is a protein coding gene, the alignment of the Cyt*b* sequences was unambiguous without any gaps. Cyt*b* sequences generated in this study were deposited in GenBank under accession numbers $$$$$$$-$$$$$$$ (see Table 3-2). In order to test the monophyly of the *Ovis* genus, we performed a Cyt*b* phylogeny of Caprinae including 28 species from 12 genera (Table 3-3).

3.3.3 Phylogeny and Sequence Analysis

Data was analyzed using Bayesian (MB), maximum likelihood (ML), and neighbour joining (NJ) methods. Bayesian analyses were performed using MrBayes V3.1.2 (Huelsenbeck & Ronquist, 2001). The Markov Chain Monte Carlo search was run with $1x10^6$generations (repeated three times), sampling the Markov chain every 100 generations, with a burn-in of 1000 trees (as detected by plotting the log likelihood scores against generation number). The most appropriate likelihood model was determined using the Akaike Information Criterion implement in ModelTest 3.07 (Posada & Crandall, 1998). ML analyses were first performed with PHYML 2.4.4 (Guindon & Gascuel, 2003), using a GTR + Γ + I model of sequence evolution. Using the best tree found by PHYML as a starting tree, heuristic ML searches were executed with PAUP* 4.0b10 (Swofford, 1998), with a tree bisection reconnection (TBR) branch swapping, and all parameter values estimated. Clade stability was estimated by non-parametric bootstrapping in 100 replicates with PHYML. NJ (Saitou & Nei, 1987) trees were constructed by using MEGA v.3.1 (Kumar *et al.*, 2004). We chose the Kimura's two-parameter distance matrix (Kimura, 1980) and the robustness of each branch was determined by a nonparametric bootstrap test with 1000 replicates and a TBR branch swapping algorithm. We used the NJ, MB and ML approaches with the same parameters as those defined above for the Cyt*b* phylogeny of Caprinae.

3.3.4 Estimation of Divergence Time

Since the likelihood ratio test rejected a global molecular clock ($P<0.05$), estimates of divergence times were obtained with the Bayesian relaxed molecular clock approach with the MULTIDISTRIBUTE program package, including ESTBRANCHES and MULTIDIVTIME (Thorne & Kishino, 2002). ESTBRANCHES was used to estimate the branch lengths of the constrained topologies and the corresponding variance-covariance matrices. The F84+Γ model was used with maximum likelihood parameters previously estimated by PAML. MULTIDIVTIME then the variance–covariance matrices produced by ESTBRANCHES were used to run a Markov chain Monte Carlo analysis to estimating mean posterior divergence times on nodes with associated standard deviation and 95% credibility interval. The Markov chain was sampled 10,000 times every 100 cycles and the burn-in stage was set to 100,000 cycles. The analysis was repeated three times. Priors were

set according to the guidelines defined in MULTIDIVTIME's manual. To determine the time separating the in-group root from the present (rttm in MULTIDIVTIME), this method needs to test different priors for the in-group age. The estimates of the age of the *Ovis* in-group ranged from 5 to 7 million years ago (MYA) according to fossil records and previous molecular data (Hartl *et al.*, 1990; Hernandez Fernandez & Vrba, 2005). Then we used six input values for the mean in-group age (rttm = 7.0, 6.4, 6.2, 6.0, 5.5 and 5.0 MYA), and the value giving the smallest standard deviations for the age of nodes was retained for further analyses. Limitations of the MULTIDIVTIME program imposed to estimate the divergence time with a sub-sample of 80 haplotypes representing the whole diversity of our dataset.

3.4 Results

3.4.1 Sequence Composition

The 235 *Ovis* individuals genotyped in this study corresponded to 102 haplotypes (Table 3-2) and the 18 individuals from GenBank corresponded to 18 other haplotypes. For the 120 haplotypes, 209 nucleotide sites (nt) over the 1140 nt utilised for the phylogenetic analyses were variable, and 148 nt were phylogenetically informative. The nucleotide frequencies were 31.56% A, 28.44% C, 12.81% G, and 27.18% T. The transition/transvertion ratio (TS/TV) was 179/45 (3.98).

Table 3-2. Wild *Ovis* samples used for Cyt*b* phylogenies

Taxon	ID	# of samples	# of Haplotype	Locality	**Accession Numbers**
Ovis orientalis					
	OgTk	31	4	Turkey	
	OgSn	10	2	Iran	
	OgAr	1	1	Armenia	
	OgBi	9	2	Iran	
	OgGa	5	3	Iran	
	OgKh	4	3	Iran	
	OgMa	12	2	Iran	
	OgMk	23	8	Iran	
	OgZa	1	1	Iran	
	OgAz	6	3	Iran	
	OgIs	8	1	Iran	
	OgBa	8	4	Iran	
Ovis orientalis musimon	OmFr	2	2	France	
Ovis vignei					
	OvGo	10	3	Kazakhstan, Turkmenistan, Iran	
	OvKa	14	4	Iran	
	OvPa	6	3	Iran	
	OvSk	4	4	Iran	
	OvTa	1	1	Iran	
	OvTm	1	1	Turkmenistan	
	OvTu	17	4	Iran	
	OvEs	4	3	Iran	
Ovis vignei blanfordi	OvbPk	3	3	Pakistan	
Ovis vignei cycloceros	OvcPk	3	3	Pakistan	
Ovis vignei punjabensis	OvpPk	3	3	Pakistan	
Ovis vignei vignei	OvvPk	2	1	Pakistan	
	OvTj	1	1	Tajikistan	
	OvKe	14	8	Iran	
	OvNo	6	4	Iran	
	OvYa	5	4	Iran	
Ovis vignei bocharensis	OvbTj	2	2	Tajikistan	
Ovis ammon					
Ovis ammon collium	OacKa	1	1	Kazakhstan	
Ovis ammon sevetzovi	OasUz	2	2	Uzbekistan	
Ovis canadensis					
Ovis canadensis canadensis	Occ	8	6	California	
Ovis canadensis nelsoni	Ocn	8	4	USA	
Ovis dalli					
Ovis dalli	Od	2	1	Canada	
Total		**235**	**102**		

Table 3-3. Taxa used for the phylogenies with respective GenBank accession numbers of Cyt*b* sequences.

DQ186288 [a]	*Bos taurus*		DQ246800 [q]	*Capra sibirica*	
AY689188 [b]	*Bos javanicus*		DQ246772 [q]	*Capra sibirica*	
AF034731 [c]	*Ammotragus lervia*		DQ514550 [h]	*Capra sibirica*	
AY397661 [d]	*Budorcas taxicolor tibe*		D32191 [j]	*Capricornis crispus*	
AY669320 [e]	*Budorcas taxicolor*		DQ459334 [l]	*Capricornis sumatrensis*	
U17868 [f]	*Budorcas taxicolor taxico*		AY669321 [e]	*Capricornis sumatrensis*	
U17867 [f]	*Budorcas taxicolor bedfor*		AY846791 [k]	*Hemitragus hylocrius*	
AB110592 [g]	*Capra aegagrus blythi*		AY846792 [k]	*Hemitragus jayakari*	
AB110593 [g]	*Capra aegagrus blythi*		AF034733 [c]	*Hemitragus jemlahicus*	
AF217255 [l]	*Capra aegagrus cretica*		U17866 [f]	*Hemitragus jemlahicus*	
DQ246781 [b]	*Capra aegagrus*		AY380560 [m]	*Myotragus balearicuspro*	
DQ514541 [h]	*Capra aegagrus*		AY356357 [l]	*Naemorhedus caudatus*	
DQ246801 [q]	*Capra caucasica*		U17861 [f]	*Nemorhaedus caudatus*	
DQ246780 [q]	*Capra caucasica*		AF190632 [n]	*Oreamnos americanusprod*	
DQ246769 [q]	*Capra cylindricornis*		AY669322 [K]	*Ovibos moschatus*	
DQ514543 [h]	*Capra cylindricornis*		U17862 [f]	*Ovibos moschatus*	
DQ514549 [h]	*Capra cylindricornis*		AF493578 [o]	*Pseudois nayaur*	
AB044309 [i]	*Capra falconeri*		AF473606 [o]	*Pseudois nayaur*	
D84202 [l]	*Capra falconeri*		AF398355 [p]	*Pseudois schaeferi*	
AB110595 [g]	*Capra hircus*		AF034726 [c]	*Rupicapra pyrenaica*	
DQ073048 [l]	*Capra hircus*		AF398353 [p]	*Pseudois schaeferi*	
AF034740 [c]	*Capra nubiana*		AB050506 [l]	*Rupicapra rupicapra tat*	
AF034738 [c]	*Capra caucasica*		AF034725 [c]	*Rupicapra rupicapra*	
DQ514552 [h]	*Capra nubiana*		AJ867266 [s]	*Ovis ammon*	Oa M33
AF242349 [l]	*Ovis ammon ammon*	Oa	AJ867260 [s]	*Ovis ammon*	Oa J17
AF242350 [l]	*Ovis ammon darwini*	Oad1	AJ867257 [s]	*Ovis ammon*	Oa J16
AF034727 [c]	*Ovis ammon darwini*	Oad2	AJ867262 [s]	*Ovis nivicola*	On1
AF034728 [c]	*Ovis dalli dalli*	Odd	AJ867263 [s]	*Ovis nivicola*	On2
AJ867275 [s]	*Ovis ammon*	Oa a2	AJ867264 [s]	*Ovis nivicola*	On3
AJ867276 [s]	*Ovis ammon*	Oa a1	AJ867261 [s]	*Ovis orientalis*	Oo J20
AJ867272 [s]	*Ovis ammon*	Oa a5	D84203 [l]	*Ovis musimon*	Om
AJ867269 [s]	*Ovis ammon*	Oa J1	AJ867267 [s]	*Ovis ammon*	Oa M23
AJ867268 [s]	*Ovis ammon*	Oa M14			

[a] (Cai et al., 2007), [b] (Hassanin & Ropiquet, 2004) [c] (Hassanin et al., 1998), [d] (Zhang et al., 2006), [e] (Ropiquet & Hassanin, 2004), [f] (Groves & Shields, 1996), [g] (Sultana et al., 2003), [h] (Pidancier et al., 2006), [i] (Mannen et al., 2001), [j] (Chikuni et al., 1995), [k] (Ropiquet & Hassanin, 2005), [m] (Lalueza-Fox et al., 2005), [n] (Hassanin & Douzery, 2000), [o] (Cao et al., 2004), [p] (Zhou et al., 2003), [q] (Kazanskaya et al., 2007), [s] (Bunch et al., 2006) [o] and [l] Unpublished.

3.4.2 Phylogenies

The phylogeny of the Caprinae subfamily including 2 samples of each *Ovis* species confirms the monophyly of the *Ovis* genus (Figure 3.2). All the other Caprinae genera are monophyletic except *Hemitragus*. *H. jemlahicus* was in the *Capra* clade, while *H. hylocrius* was close to the *Ovis* clade. The position of *H. jayakari* was not well resolved.

When focusing on the *Ovis* genus, the three independent Bayesian analyses converged on similar log-likelihood scores and reached stationarity before 50,000 generations (plot not shown). The consensus topologies of the three runs were identical (Figire 3-3). The two other phylogenetics methods (ML and NJ) gave the same topology (data not shown, bootstrap values given in Figire 3-3 on the consensus Bayesian tree). Several monophyletic groups supported by high bootstrap values are distinguished. A first Pachyceriform group is composed of the Snow sheep (*O. nivicola*) and the two American sheep (*O. canadensis* and *O. dalli*). The other Eurasian sheep are divided in to the Argaliform group (Argali *O. ammon*) and the moufloniform group. This last group is subdivided into two monophyletic taxa, the Urial (*O. vignei*) and the mouflons (*O. orientalis*). The European mouflon (*O. musimon*) is clearly included in the *O. orientalis* clade (Figire 3-3).

3.4.3 Estimations of Divergence Times

Using MULTIDIVTIME, the age of the in-group giving the smallest standard deviations for the age of nodes was 6.40 ± 0.05 MYA. This value was used to calibrate the Bayesian tree for estimating the divergence times under a relaxed molecular clock approach. The divergence between the American wild sheep (*O. dalli* and *O. canadensis*) from *O. nivicola* occurred about 4.21 ± 0.95 MYA. At about the same time (4.66 ± 0.83 MYA) the Argali (*O. ammon*) diverged from the other Eurasian groups. Then the Mouflon (*O. orientalis*) and the Urial (*O. vignei*) diverged about 3.55 ± 0.89 MYA. The two American species began to diverge around 2.65 ± 0.95 MYA (Figire 3-3).

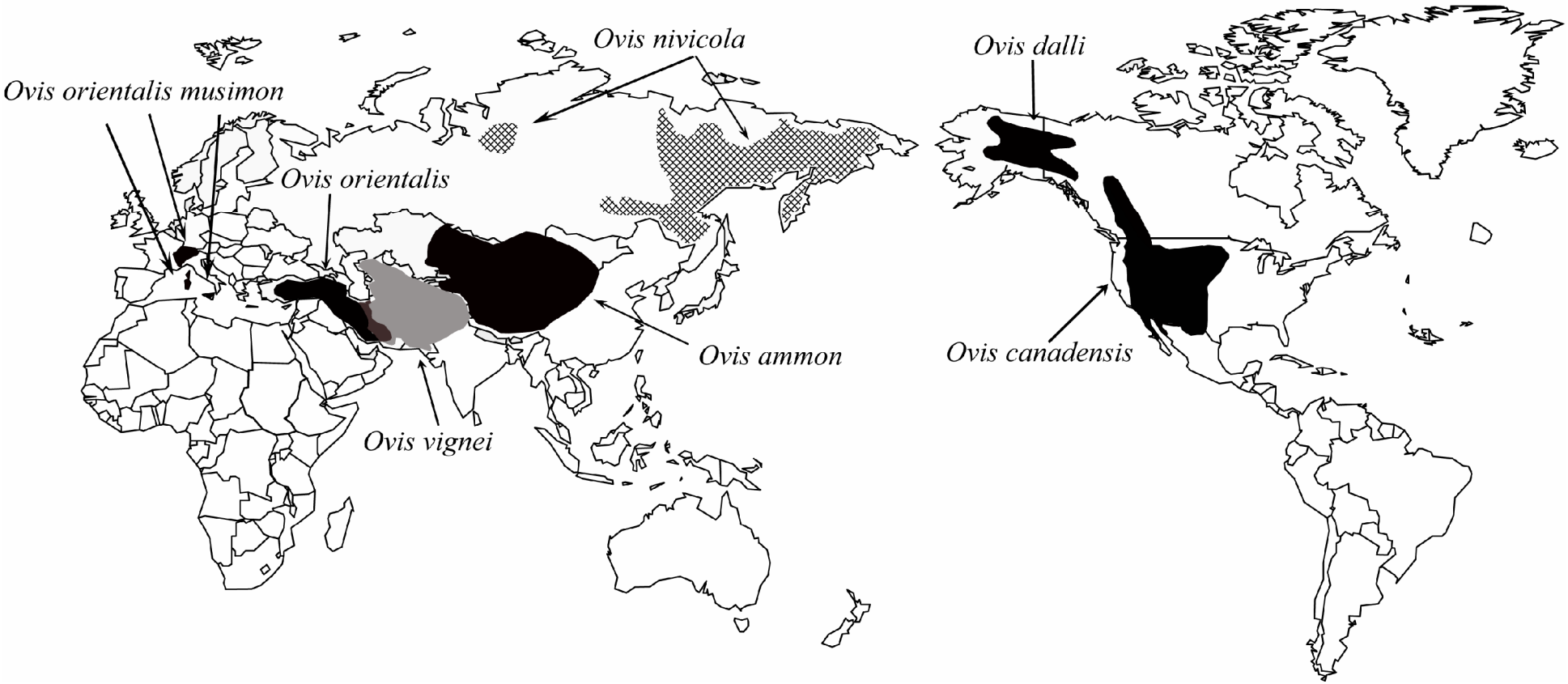

Figure 3-1. Approximate geographic distributions of wild *Ovis*: Argali (*O. ammon*), Snow sheep (*O. nivicola*), Dall sheep (*O. dalli*), Bighorn (*O. canadensis*), Urial (*O. vignei*), Asiatic mouflon (*O. orientalis*) and European mouflon (*O. orientalis musimon*).

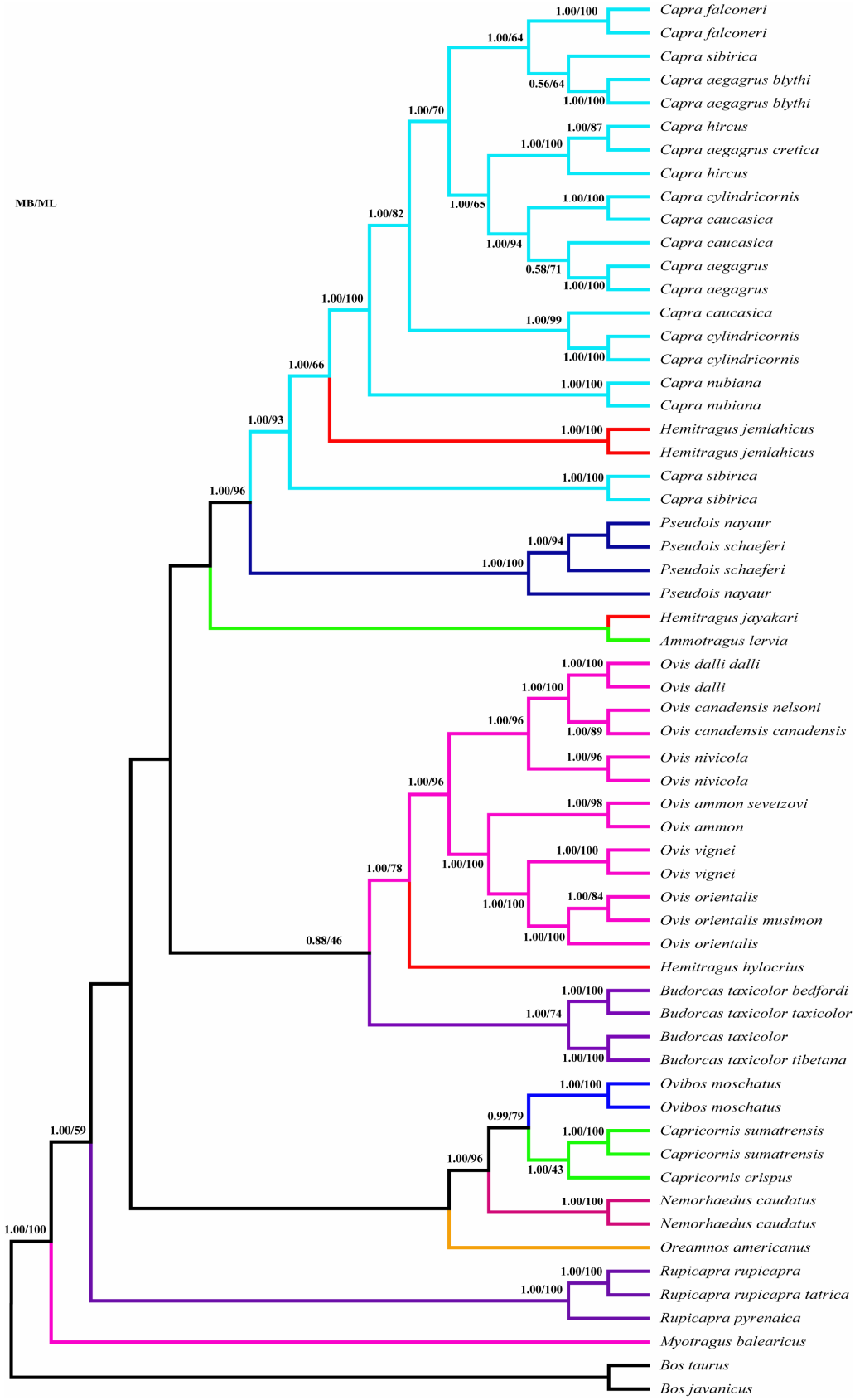

Figure 3-2. Bayesian and Maximum likelihood phylogeny of Caprinae based on complete cytochrome *b* sequences. Outgroup includes *Bos taurus* and *B. javanicus.*

3.5 Discussion

The use of morphological characters alone (horn morphology and coat pattern) is not adequate for inferring the evolutionary history and classification of the wild *Ovis*, and genetic data such as chromosome number did not suffice to solve all the problems (Shackleton & Lovari, 1997; Valdez *et al.*, 1978). The concomitant use of gene phylogenies is thus necessary. Although the molecular tool has been commonly used for phylogenetic studies for more than two decades, there has been no study of wild sheep based on large samples from their entire distribution area until now. The present Cyt*b* phylogeny gives new insights into *Ovis* evolution and classification.

3.5.1 Evolutionary History of Wild Sheep

The monophyly of the genus *Ovis* has been established in phylogenies based on molecular data (Hassanin & Douzery, 1999; Lalueza-Fox *et al.*, 2005; Ropiquet & Hassanin, 2004; Ropiquet & Hassanin, 2005), karyotype (Huang *et al.*, 2005) or combining morphological, ethological and molecular information (Hernandez Fernandez & Vrba, 2005). However, none of these studies included all the wild species of the *Ovis* genus and some authors have proposed that it could be paraphyletic (Groves & Shields, 1996; Groves & Shields, 1997). The present Cyt*b* phylogeny of Caprinae confirms the *Ovis* monophyly based on a sampling representative of the diversity of each wild sheep species. The phylogenetic proximity of Asiatic species to the *Capra, Hemitragus* and *Pseudois genera*, and the ancestral position of the Asiatic *Ovis* species are in favour of an Asiatic sheep ancestor. This is in accordance with the fossil record and karyotypic studies that support an Eurasian origin of the genus (Bunch *et al.*, 2000; Bunch *et al.*, 2006). The American sheep (*O. canadensis* and *O. dalli*) are monophyletic and form a monophyletic group with the Siberian Snow sheep (*O. nivicola*). This supports the hypothesis of the migration of Asiatic sheep to North America through North-Eastern Asia and the Bering Strait. This came with a differentiation of the American sheep from *O. nivicola* about 4 MYA and a divergence times between *O. dalli* and *O. canadensis* of about 2.6 MYA. In Asia, the divergence between *O. ammon* and the *O. gemilinii/vignei* group occurred about 4.6 MYA and *O. vignei* diverged from *O. orientalis* about 3.5 MYA. These values are different from those given by Bunch *et al.* (2006) due to a difference in the calibration date of the tree. The

divergence time of 2.5 MYA for the *Ovis* genus used by Bunch *et al.* (2006) was based on the *Ovis* fossil record. It is not in accordance with the more recent estimations combining fossil and molecular data that we used for calibration (Hartl *et al.*, 1990; Hernandez Fernandez & Vrba, 2005). This difference may be related to the lack of a good *Ovis* fossil record because of the bad conditions for fossilization in the mountain regions that wild sheep inhabit (Bunch *et al.*, 2006).

O. ammon, *O. nivicola*, *O. dalli* and *O. canadensis* form monophyletic taxa that were supported by robust bootstraps values. This confirms their species status that has been accepted by all recent classifications (Nadler *et al.*, 1973; Shackleton & Lovari, 1997; Valdez, 1982). A previous Cyt*b* phylogeny found *O. ammon* and *O. nivicola* polyphyletic (Bunch *et al.*, 2006). This is not the case in our phylogeny, which included data from this previous study, except for 4 haplotypes (3 *O. ammon* and 1 *O. nivicola*). The distribution of mutations along the sequences clearly shows that these four haplotypes are chimeric sequences mixing *O. nivicola* and *O. ammon*.

The Asiatic mouflon and the urial are either classified as a single species (*O. orientalis*) or as two separate species (*O. orientalis* and *O. vignei*). Differences in horn morphology and coat (presence of a throat bib in the urial and not in the mouflon), and mainly in chromosomes number (2n=58 in the Urial and 2n=54 in the mouflon) support the existence of two species (Nadler *et al.*, 1973). The occurrence of hybrid populations with intermediate morphologies and chromosomes numbers (all possibilities between 2n = 54 and 58) would support the existence of a single species (Valdez, 1982). The Cyt*b* phylogeny shows that the individuals identified as mouflon and urial form two monophyletic groups that are strongly supported by high bootstrap values. According to the origin of their mitochondrial DNA, individuals from hybrids populations appear either in the *vignei* or in the *orientalis* taxon, independently from their geographic origin. Thus these two groups clearly form two distinct evolutionary lineages that are hybridizing in their contact zones. Considering these two taxa as distinct species would be more coherent with the morphological and genetic differences between them, their past evolutionary divergence and the occurrence of a restricted hybrid zone. Understanding the functioning of the hybrid zone and measuring the degree of introgression between species requires the study of nuclear markers and remains to be done.

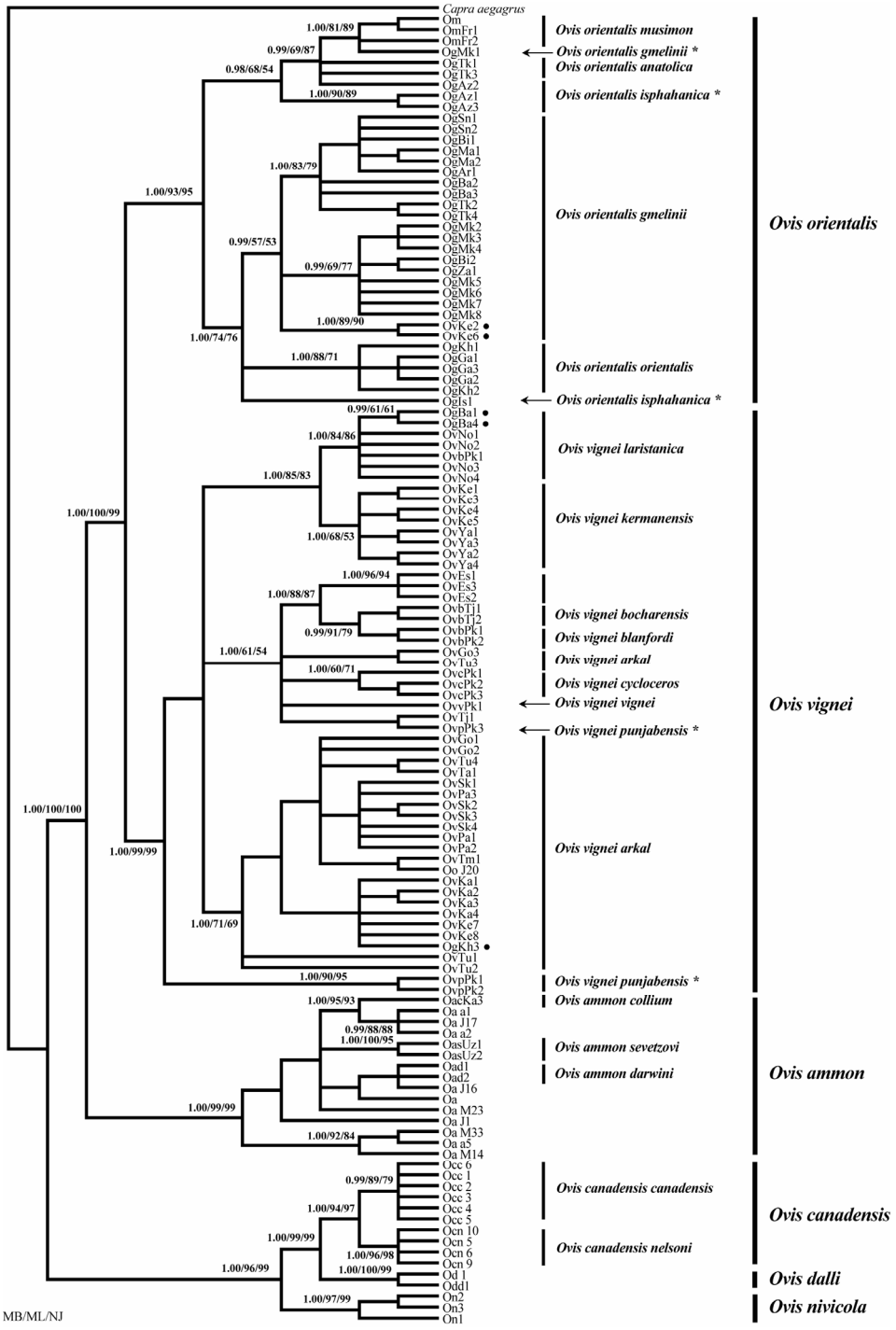

Figure 3-3. Phylogenetic relationships within the *Ovis* genus based on complete cytochrome *b* sequences using the MB, ML and NJ methods, (●): haplotypes from the hybrid zone between *O. orientalis* and *O. vignei*, (★): polyphyletic subspecies.

Our sampling allows us to test the monophyly of several taxa considered as subspecies of *O. vignei* and *O. orientalis* (Shackleton & Lovari, 1997; Valdez *et al.*, 1978). The Severtzov's Urial from Uzbekistan that has been recognised as a subspecies of *O. orientalis* (Shackleton & Lovari, 1997), appears as a subspecies of *O. ammon* on the Cyt*b* phylogeny. This is in accordance with previous results, which classified this subspecies in the Argali group on the basis of morphological and karyotypic criteria (Bunch *et al.*, 1998). At least five of the other subspecies (i.e., *O. orientalis gmelini*, *O. orientalis isphahanica*, *O. vignei blanfordi*, *O. vignei arkal* and *O. vignei punjabensis*) are not monophyletic. Considering the overlap in the geographic distribution of these subspecies, this may result from gene flows between populations. We cannot exclude that the other subspecies, which appear to be monophyletic with the present samples, are in fact polyphyletic. Studies based on nuclear DNA with wider sampling are needed for measuring gene flows and understanding these phenomena.

According to the Cyt*b* phylogeny, *O. musimon* clearly appears to be within the *O. orientalis* clade. Thus it should not be considered as a separate species as stated by Nadler *et al.* (1973), but as a subspecies of *O. orientalis* as recognized by other authors (Valdez, 1982; Wilson & Reeder, 1993). *O. orientalis musimon* represents the only European wild *Ovis*, and should now be considered as a wild remnant of the first domestic sheep that entered Europe based on archaeological (Poplin, 1979; Vigne, 1988) and genetic evidence (Bruford & Townsend, 2006).

The polyphyly of most of the subspecies previously defined on morphological and geographical criteria question the use of these subspecies as conservation units. This is especially true for the hybrid populations between *O. vignei* and *O. orientalis* in Iran. By pointing out the high diversity of wild sheep and the phylogenetic relationships between taxa, this study also has implications in the conservation biology of a genus where 13, 7 and 3 subspecies are respectively considered as vulnerable, endangered and critically endangered in the IUCN red list (IUCN, 2006).

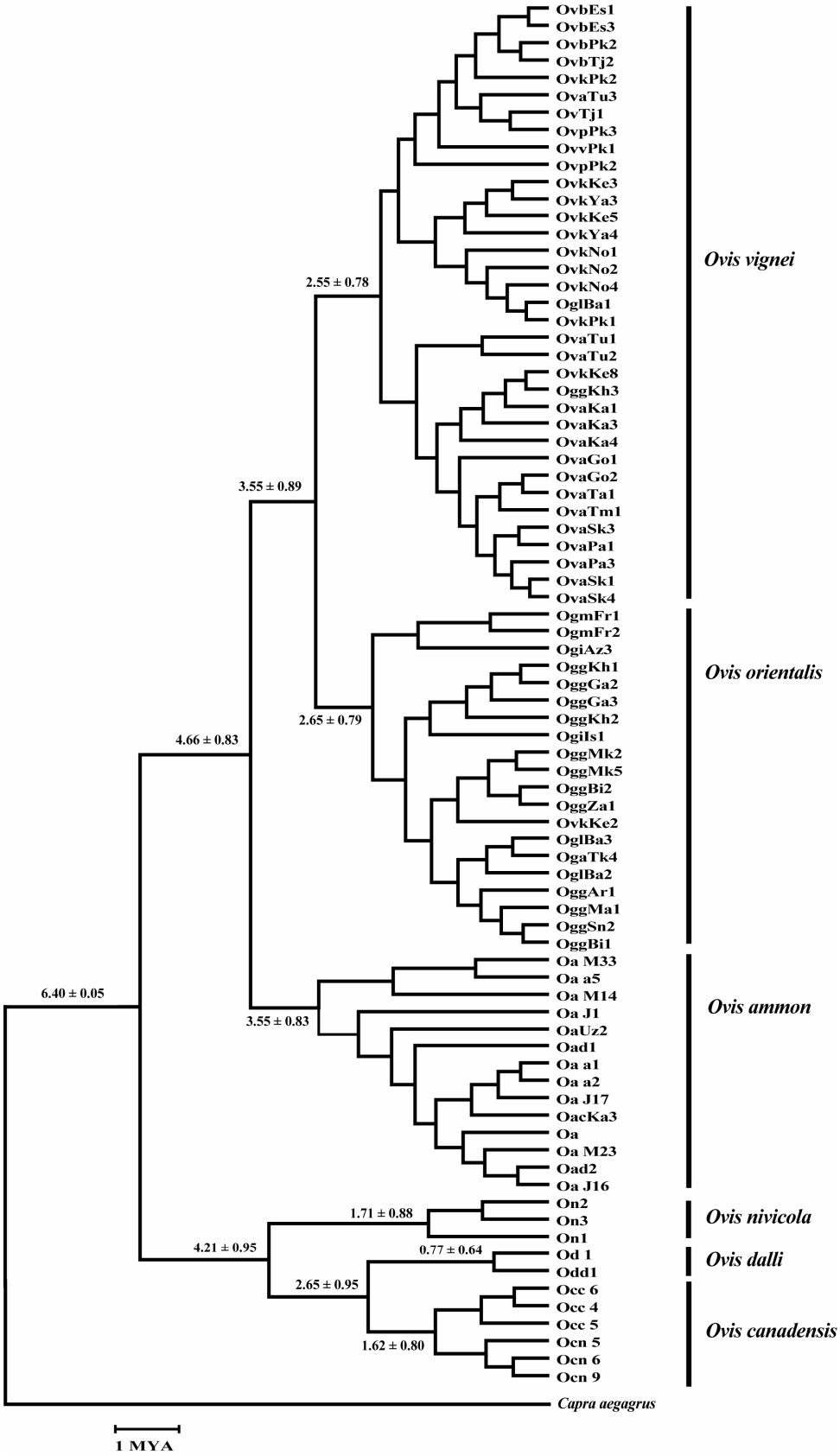

Figure 3-4. Chronogram from Bayesian dating analysis

3.6 Acknowledgements

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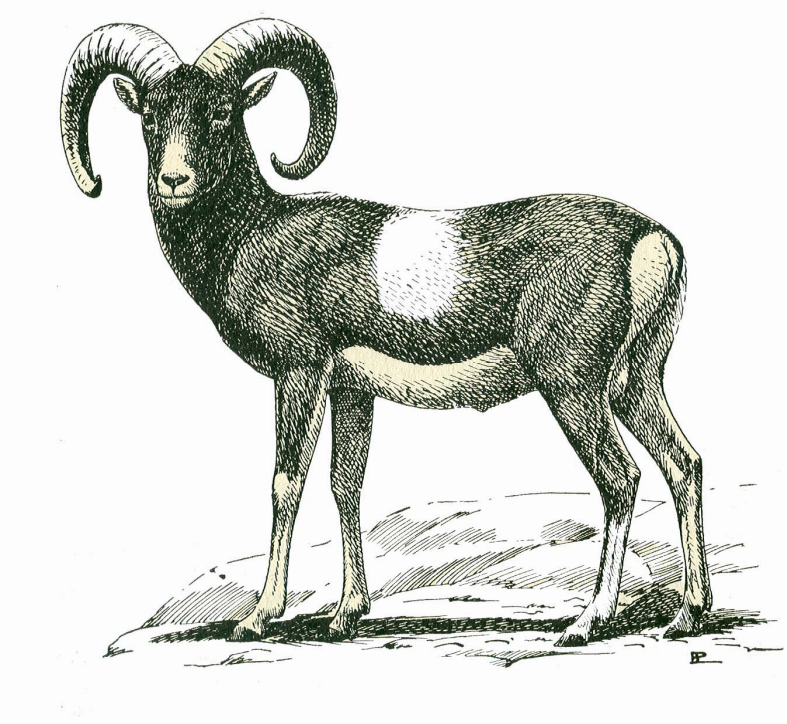

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Chapter 4

The Origin of Domestic Sheep

4. The Origin of Domestic Sheep

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4.1 One-sentence summary:

Analyses of genetic diversity in sheep (*Ovis aries*) and its wild relatives demonstrates that it has been domesticated from the Asiatic mouflon (*Ovis orientalis*) over a large area in the Anatolian/Zagros mountains without a concomitant genetic bottleneck.

4.2 Abstract

The origin of domestic sheep (*Ovis aries*) is controversial, with several putative wild ancestors and two potential domestication areas implicated. A phylogeny based on an extensive sampling of modern sheep and its plausible ancestral species demonstrates that the Asiatic mouflon (*O. orientalis*) is the sole ancestor of the domestic form. Comparison of mitochondrial (mt)DNA diversity in 130 domestic sheep with that of 140 Asiatic mouflon from across its distribution area localizes the cradle of domestication between Eastern Anatolia and the Zagros mountains, clearly excluding the Lower Indus Valley and more Eastern Asian regions. A large element of the wild mitochondrial and nuclear DNA diversity has been captured during domestication, implying a large effective population size at the time of domestication, contrary to current domestication paradigms.

4.3 Main Text

The Neolithic transition from hunter-gatherer to a sedentary lifestyle irreversibly disrupted human socio-cultural organizations. It was related to a major demographic increase (Bocquet-Appel, 2002) *(1)* and corresponds with the domestication of plant and animals that led to pastoralism. Together with the goat, the sheep was probably amongst the first livestock species to be domesticated and transported across the globe (Harris, 1996; Vigne *et al.*, 2005a; Zeder & Hesse, 2000) *(2-4)*. The earliest evidence for the presence of domesticated sheep has been found in the Taurus mountains, Southeastern Anatolia ca. 10,500 cal. B.P. (Peters *et al.*, 2005) *(5)*. Recent studies have invalidated the hypothesis of a local early domestication of sheep in the Levant, where the sheep was instead probably introduced from the North during the 9th millennium (Bar Yosef, 2001; Horwitz & Ducos, 1998) *(6, 7)*. According to most recent studies, the early appearance of domestic sheep at the turn of the 10-9th millennia in the Middle Euphrates Valley, Northern Levant (Damascus) and Cyprus, results from animal transportations from the Taurus Mountains (Legge, 1996; Saña Seguí, 1999; Vigne *et al.*, 2003; Vigne *et al.*, 2000) *(8-11)*. In the Zagros area, sheep seem to have also been introduced from Anatolia during the course of the 9th millennium (Zeder, 2003; Zeder, 2005) *(12, 13)*. However, some evidence suggests that sheep could have been locally domesticated in the Lower Indus valley during the early 7th/late 8th millennium BP (Meadow, 1996) *(14)*. Based on archaeological and genetic studies, several wild Asiatic species have been proposed to be the ancestor of domestic sheep (Hiendleder *et al.*, 2000; Nadler *et al.*, 1973; Pedrosa *et al.*, 2005; Reed, 1984; Zeuner, 1963) *(15-19)*. These are the Argali *Ovis ammon*, the Asiatic Mouflon *O. orientalis* and the Urial *O. vignei* (Fig. 1A and 2). Archaeozoological research has suggested the elimination of *O. ammon* as well as *O. vignei* as potential ancestors (Clutton-Brock, 1981; Uerpmann, 1987; Uerpmann & Frey, 1981) *(20-22)*, but fluctuations in the nomenclature between *O. orientalis* / *vignei* suggest some credibility for the hypothesis of a contribution from *O. vignei*. Genetic data based upon the occurrence of highly divergent mitochondrial haplogroups in domestic populations suggest that multiple domestication events could have occurred, even involving multiple taxa (Bruford & Townsend, 2006; Hiendleder *et al.*, 2002; Meadows *et al.*, 2007; Pedrosa *et al.*, 2005; Tapio *et al.*, 2006) *(19, 23-26)*.

MtDNA has been extensively used to describe the genetic diversity of domestic animals and to assess their origin and history (Bruford *et al.*, 2003; Zeder *et al.*, 2006) *(27-28)*. We used the complete mitochondrial cytochrome *b* (Cyt*b*) gene sequence to compare the mtDNA diversity of wild and domestic sheep. First, we aimed to test the archaeozoological hypothesis of a unique *O. orientalis* ancestor for the domestic sheep by analyzing the phylogenetic relationships between 130 domestic sheep with 267 individuals of the three putative ancestral taxa from 55 localities covering most of their distribution range (Fig. 1A). Second, we attempted to localize the domestication center(s) by finding the wild populations of the putative ancestral species that are genetically closest to the domestic populations. Third, we investigated the occurrence of a 'pre-domestication step' by looking for a genetic signature of expansion in the wild sheep from which the domestics originate. Such a pre-domestication step would correspond to an initial phase of sustainable management of wild flocks, as been shown for the goat (Naderi *et al.*, 2008) *(29)*. Finally, we tested the possible occurrence of a demographic bottleneck at the time of domestication by estimating the number of mtDNA haplotypes captured and the diversity of nuclear loci.

The mtDNA phylogenetic relationships among *O. aries* and its three putative ancestral species clearly show that *O. orientalis* is the sole wild ancestor of all modern domestic sheep (Fig. 2). Sequencing of 12 nuclear genes for 84 individuals belonging to *O. orientalis*, *O. vignei*, and *O. aries* does not contradict the mtDNA results, but is inconclusive due to the large retention of ancestral polymorphisms between *O. orientalis* and *O. vignei* (See Supplementary Information). The exclusion of *O. vignei* and *O. ammon* from the origin of the modern domestic sheep precludes any contribution to the Lower Indus Valley (Meadow, 1996) *(14)* or even a more easterly location as the origin of any sampled domestic sheep lineage.

The clade containing *O. aries* and *O. orientalis* is divided into two clusters both containing haplotypes from wild and domestic sheep (Fig. 3A). This partition confirms divergence between the domestic haplotypes of the A/B haplogroups from those of the C/E haplogroups (Meadows *et al.*, 2007) *(26)*. The D haplogroup identified from mtDNA control region sequences (Tapio *et al.*, 2006) *(25)* was not found when analyzing Cyt*b* sequences. However, the geographical distribution of the wild haplogroups (Fig. 3B) indicates that there is no concordance between the divergence of mtDNA haplotypes and currently recognized sub-species of *O. orientalis* that have been defined on morphological and geographical criteria, such as *O. o. orientalis* or *O. o. isphahanica*.

The geographic distribution of the mtDNA haplogroups in the modern Asiatic mouflon (Fig. 3B) suggests three possible contiguous centres for early sheep domestication: Central Anatolia (A haplogroup), Northern Zagros (A, B and E haplogroups) or Central Zagros (C haplogroups). However, genetic data are lacking on the now extinct mouflon populations which are presumed to have existed in the upper Euphrates and Tigris Valleys (Eastern Anatolia) at the time of domestication. Based on archaeozoological data, this area is suspected to be where sheep domestication began and from whence it spread both to the Central Zagros and to Central Anatolia. It addition, it is not possible to distinguish whether the presence of some haplotypes similar to domestic sheep in modern *O. orientalis* populations is either the evidence that the latter gave rise to the domestic haplogroups, or is the result of later introgression from the domestic stock into the wild population. By combining archaeological and genetic data, the most probable scenario is that the A/B and the C/E haplogroups were domesticated in the Eastern Anatolia and in the Northern/Central Zagros, respectively. The domestication of the A and B haplogroups in Eastern Anatolia is supported by the fact that only these two haplogroups are present in Europe in the domestic species, and that Europe has most likely been colonized by populations located at the western side of the domestication center(s). Furthermore, these two haplogroups are closely related in the phylogenetic tree (Fig. 3A), and are thus likely to have been geographically proximate. The C and E haplogroups are only found in domestic sheep from Asia, together with the A and B haplogroups. This suggests an eastern location within the domestication center(s), in Northern and possibly Central Zagros. Finally, the fact that the A and B haplogroups are present in all populations of domestic sheep today suggests that they were domesticated and spread first, before the C and E haplogroups. Such a scenario is fully consistent with archaeological data that suggest Eastern Anatolia as the most ancient evidence of sheep domestication, and a single subspecies of the Asiatic mouflon (*O. orientalis gmelini*) domesticated.

For goats, a phase of sustainable management of wild flocks - a 'pre-domestication' step - took place before the true domestication and has been characterized by a signature of population size increase in the wild ancestors that gave rise to the domestics (Naderi *et al.*, 2008) *(29)*. Such a population expansion is still detectable today when analyzing mtDNA polymorphism. Do sheep also exhibit evidence for such a pre-domestication step? Our data do not provide greater evidence for demographic expansion in wild populations closest to domestic sheep as opposed to other wild *Ovis*. Thus, it does not seem that wild *Ovis* flocks underwent a strong population expansion before the true domestication of sheep. However,

this does not mean that sheep domestication occurred at a reduced spatial (hence genetic) scale. It appears that a very high amount genetic diversity has been captured during domestication, both for mitochondrial and nuclear DNA. For mtDNA, the analysis of the current polymorphism in sheep suggests that more than 200 haplotypes were subsumed before the geographic spread of domestic sheep outside the range of its ancestor *O. orientalis* (See Supplementary Information). Such a result is consistent with the relatively high polymorphism observed today in sheep Cyt*b*, and with the known evolutionary rate of this gene (4% sequence divergence per million years (Irwin *et al.*, 1991)) *(30)*. It is interesting to note that the same Cyt*b* sequence (Rezaei *et al.*, 2007) *(31)* have been found in some Portugese domestic sheep, as well as in the European mouflon that became feral about 5000 years ago in Corsica (Poplin, 1979) *(32)*. Thus, no mutation occurred with a divergence of at least 5000 years, suggesting very few mutations since the domestication, and supporting the high number of initial haplotypes in proportion to the polymorphism the main haplogroups of domestic sheep. In common with goats and horses (Jansen *et al.*, 2002; Naderi *et al.*, 2008) (29, 33), such a high number of initial haplotypes is not compatible with the occurrence of a bottleneck during sheep domestication.

By combining genetic data on domestic sheep and its putative wild ancestors with archaeozoological data, we are able to propose a realistic scenario accounting for the origin of this domestic species. Only a single subspecies of the Asiatic mouflon (*O. orientalis gmelini*) appears to have been involved in the domestication process, in Eastern Anatolia first and in Northern Zagros probably slightly later. A domestication center in the Indus Valley or in another eastern location is not consistent with our results, because all the domestic mtDNA haplotypes fall within the Asiatic mouflon clade that is monophyletic. Except for the absence of a pre-domestication step, sheep domestication shows many similarities with goat domestication. First, they occurred in the same regions. Second, the domestication was a large-scale process, without substantial demographic bottlenecks, involving the capture of many mtDNA haplotypes and a large proportion of the nuclear genome of the wild ancestor. Third, the wild ancestor is not extinct and represents a valuable genetic resource for the sustainable management of the domestic species.

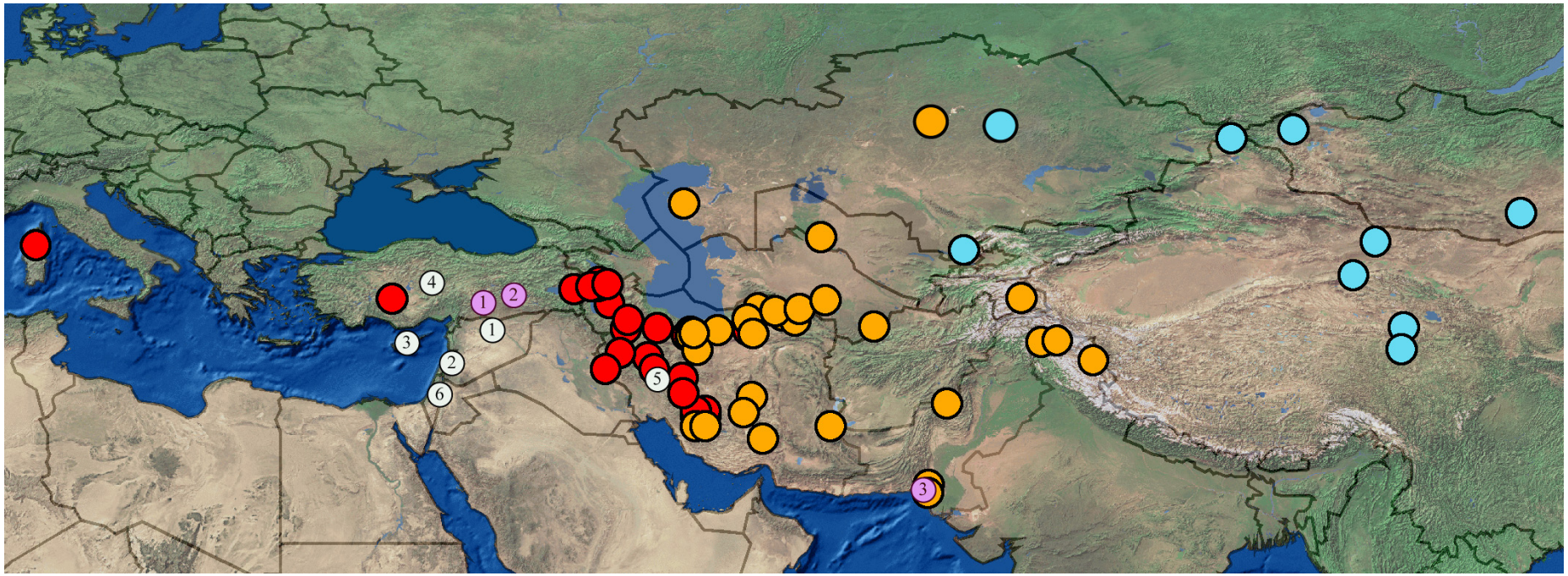


Figure 4-1 Figure 1 Geographic distributions of the Asiatic mouflon (*Ovis orientalis*, red dots), the Urial (*O. vignei*, yellow dots), and the Argali (*O. ammon*, green dots), the three putative ancestral species of domestic sheep. Blue dots : Site with putative local domestication of sheep: 1, Nevalı Çori (Turquey, ca. 10,500 BP), 2, Cayönü (Turkey, 10,200-10,000 BP), 3, Mehrgarh (Pakistan, ca. 8000 BP). Violet dots : **Some sites with early evidence of domestic sheep transfer:** 1, Tell Halula (Syria, ca. 9,700 BP), 2, Aswad (Syria, 10,300-10,000 BP), 3, Shillourokambos (Cyprus, ca. 10,000 BP), 4. Aşıklı (Turkey, 10,000-9,800 BP), 5. Tapeh Guran (Iran, 9500-9000 BP), 6. Ain Ghazal (Israel, ca. 9,500-9,000 BP)

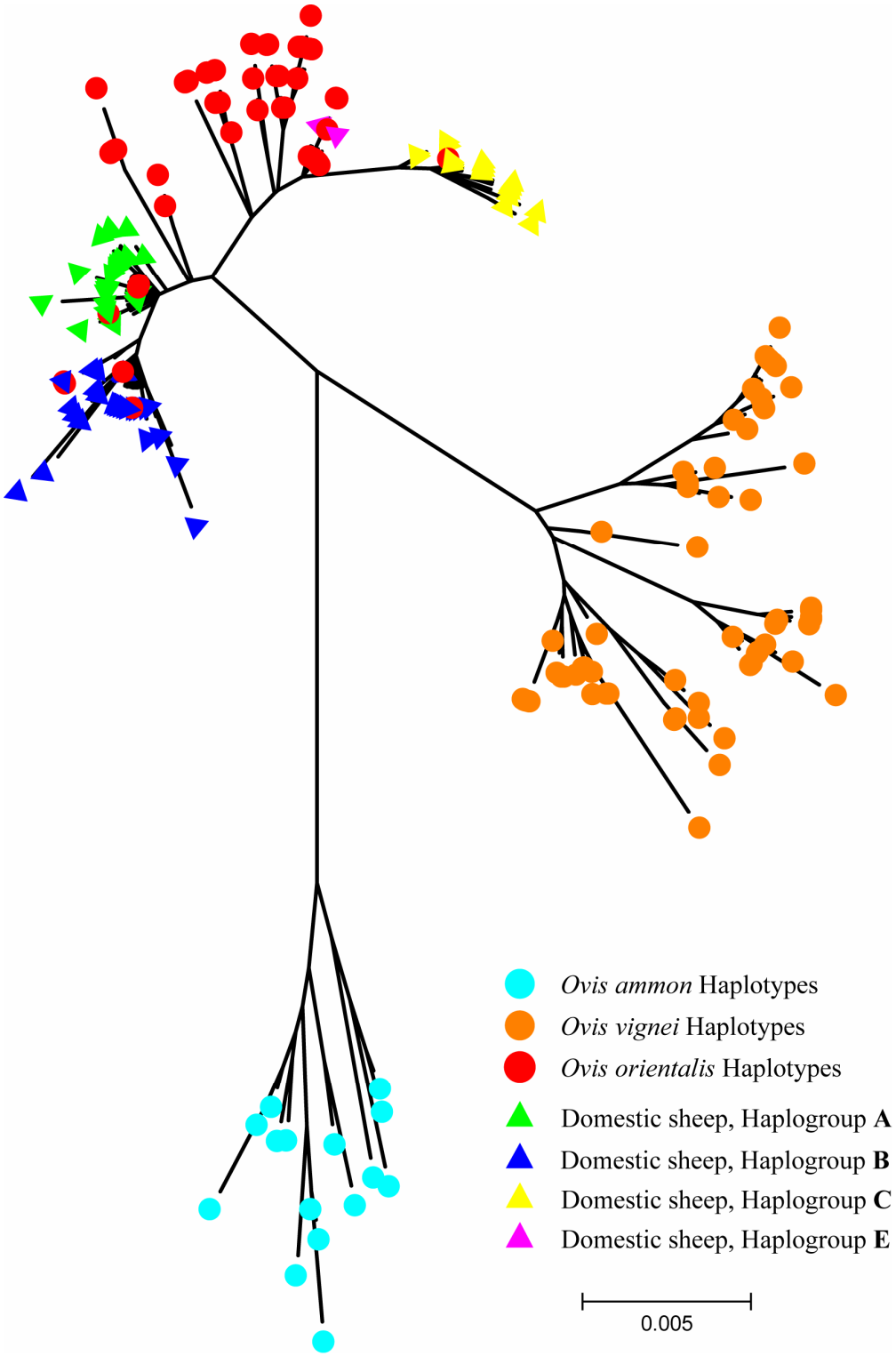
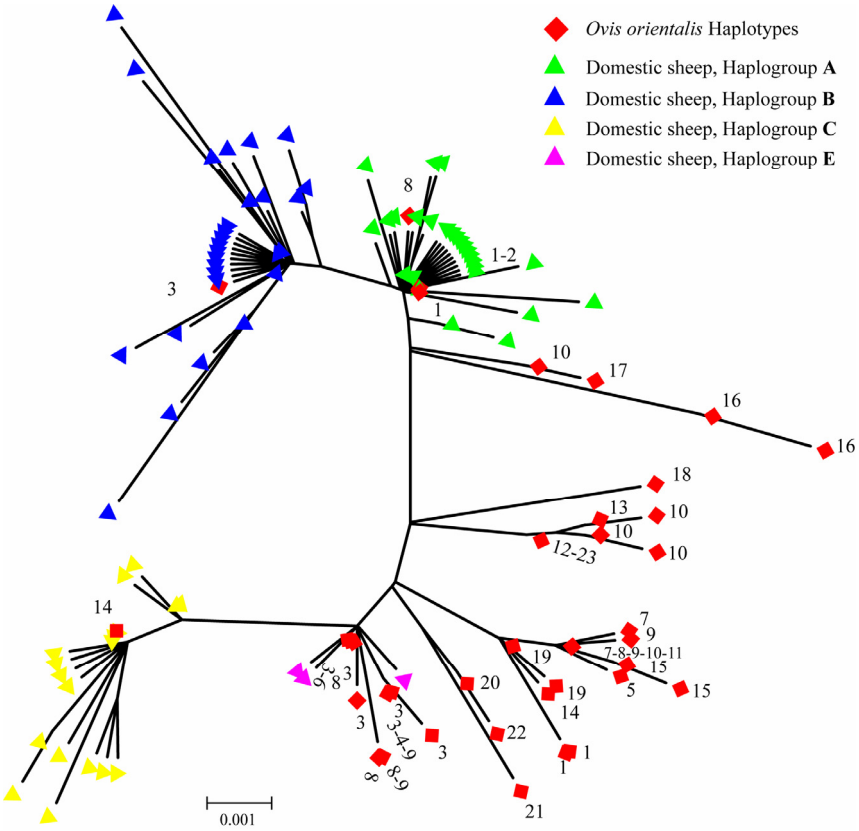


Figure 4-2 Origin of the domestic sheep inferred from mitochondrial DNA polymorphism. Phylogenetic relationship of domestic sheep mitochondrial DNA compared with the three putative ancestral species, the Asiatic mouflon, the Urial, and the Argali. This tree was obtained with the neighbour-joining method (Saitou & Nei, 1987) *(34)*, and shows that domestic sheep only originated from the Asiatic mouflon.

A



B

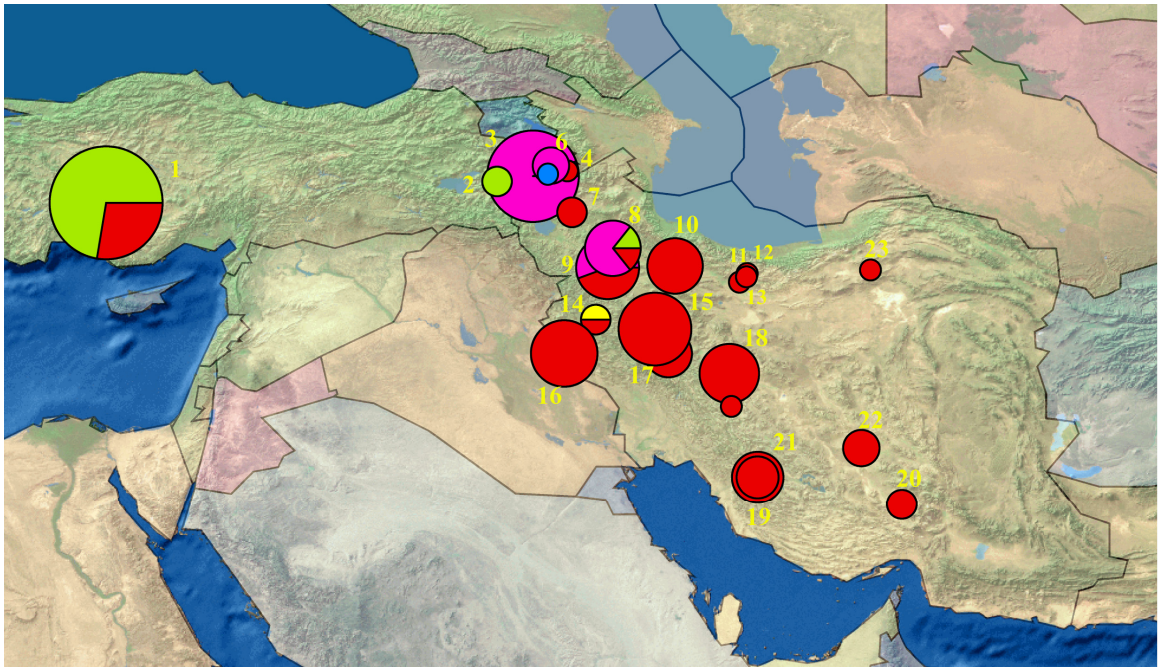


Figure 4-3 A: Phylogenetic relationship among the different haplotypes of the Asiatic mouflon. A few domestic sheep haplotypes characterizing the different haplogroups have been also included in this tree. The numbers correspond to the sampling locations in Fig. 3B. B: Geographic distribution of the different mitochondrial DNA haplotypes of the wild *orientalis* mouflon. The colours of the different haplogroups are the same as in Fig. 3A.

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4.5 Supplementary Information

4.5.1 Sampling

The 267 wild sheep (*Ovis* genus) samples include 140 *O. gmelinii*, 111 *O. vignei* and 16 *O. ammon* from 45 geographic localities representing most of their distribution area in Asia. Most of samples were obtained using a non-invasive method (Taberlet & Luikart, 1999) (S1). Fresh faeces were collected in the field, after observation of the animal from a distance to ensure the identification of the sample. Two samples were collected for each individual and preserved with two methods (silica gel and ethanol 96%). Some samples comprised skin and muscle obtained from hunter kills and carcasses. Because of possible hybridization in captivity, no samples were considered from zoos in this study. All wild sheep samples used for mtDNA analysis are listed in table 4-1. In addition, we collected 83 domestic sheep tissue samples from different countries (Table 4-1). We obtained 46 domestic and 14 different wild samples sequences from GenBank (Table 4-2). A total of 68 wild and domestic sheep were sampled from the major part of the distribution of three wild species of *Ovis* in Asia. The samples consisted of faeces, skin and muscle (Table 4-3).

4.5.2 DNA extraction

The whole genomic DNA was extracted from fecal samples after 20 minutes in washing buffer (Tris-HCl 0.1 M, EDTA 0.1 M, NaCl 0.1 M, N-lauroyl sarcosine 1%, pH 7.5-8.0), using DNAeasy extraction blood kit (Qiagen) following the manufacture's protocol except for the incubation with protease (2 hours at 56°C with 55 µl (> 33 mAU/ ml) of protease). For tissue samples, total DNA was extracted using the tissue extraction kit QIAamp Animal Tissue kit (Qiagen) following the manufacture's instructions.

4.5.3 DNA amplification

The complete mitochondrial Cyt*b* gene was amplified with two pairs of primers (Pedrosa *et al.*, 2005) (S2); (Table 4-4). PCR reactions were performed in a final volume of 25 µl containing two µl of DNA, 1 µM of each primer, 1x PCR buffer, 200 µM of each dNTP, 1.5 mM MgCl2, and one unit of AmpliTaq Gold polymerase (Applied Biosystems).

PCR was performed according to the following protocol: initial denaturation, 95°C, 10 min; then for 35-40 cycles, denaturation, 95°C, 30 s; annealing, 55°C or 60°C (for CYTB_F/ CYTB_IN_R and CYTB_IN_F/CYTB_R, respectively), 30 s; extension, 72°C, 1 min; a final extension, 7 min, 72°C. PCR products were purified using the Qiaquick kit (Qiagen) following the manufacturer's instructions.

Twelve different nuclear loci were selected, each situated in different genes. For each locus, primers were newly designed (table S3). The PCR reactions for each locus were performed in a final volume of µl containing two µl of DNA, 0.5 µM of each primer, 1x PCR buffer, 200 µM of each dNTP, 1.5 mM MgCl2, and one unit of AmpliTag Gold polymerase (Applied Biosystems). PCR was performed according to the following protocol: initial denaturation, 95°C, 10 min; then for 30-35 cycles, denaturation, 95°C, 30 s; annealing, depending on the locus and primers (table S3), 30 s; extension, 72°C, 1 min; a final extension, 7 min, 72°C. PCR products were purified using the Qiaquick kit (Qiagen) protocol following the manufacturer's instructions.

4.5.4 Sequencing

Purified PCR products were used as template in 20 µl sequencing reactions involving the BigDye Terminator Cycle Sequencing kit version 3.1 (Applied Biosystems) and analyzed on an ABI Prism 3700 semi-automated DNA analyser (Applied Biosystems) using the POP 7 polymer. SeqScape 2.5 (Applied Biosystems) was used to reconcile chromatograms of complementary fragments and to align sequences across taxa. As Cyt*b* is a protein-coding gene, alignment of the Cyt*b* sequences was unambiguous without any gaps. Cyt*b* sequences generated in this study were deposited in GenBank under accession numbers $$$$$$$-$$$$$$$ (table S1). For 12 nuclear genes we used the same methods of sequencing, the sequences were deposited in GenBank under the accession numbers $$$$$$$-$$$$$$$ (Table 4-2).

4.5.5 Phylogenetic analysis

As Cyt*b* is a protein-coding gene, the alignment of the Cytb sequences was unambiguous without any gaps. For nuclear genes, sequences were aligned with MEGA v.3.1 (Kumar *et al.*, 2004) (*S3*). After alignments, data were analyzed using Bayesian (MB), maximum likelihood (ML), and neighbour joining (NJ) methods. Bayesian analyses

were performed using MrBayes V3.1.2 (Huelsenbeck & Ronquist, 2001) (*S4*). The Markov Chain Monte Carlo search was run with 1x106generations (repeated three times), sampling the Markov chain every 100 generations, with a burn-in of 1000 trees (as detected by plotting the log likelihood scores against generation number). The most appropriate likelihood model was determined using the Akaike Information Criterion implement in ModelTest 3.07 (Posada & Crandall, 1998) (*S5*). ML analyses were first performed with PHYML 2.4.4 (Guindon & Gascuel, 2003) (*S6*), using a GTR + Γ + I model of sequence evolution. Using the best tree found by PHYML as a starting tree, heuristic ML searches were executed with PAUP* 4.0b10 (Swofford, 1998) (*S7*), with a tree bisection reconnection (TBR) branch swapping, and all parameter values estimated. Clade stability was estimated by non-parametric bootstrapping in 100 replicates with PHYML. NJ (Saitou & Nei, 1987) (*S8*) trees were constructed by using MEGA v.3.1. We chose the Kimura's two-parameter distance matrix (Kimura, 1980) (*S9*) and the robustness of each branch was determined by a nonparametric bootstrap test with 1000 replicates and a TBR branch swapping algorithm.

The nuclear data were analysed at the first separately for each gene. In addition, the combination of all sequences were analysed with NJ methods. NJ (Saitou & Nei, 1987) (*S8*) trees were constructed by using PAUP* 4.0b10 (Swofford, 1998) (*S7*) and MEGA v.3.1 (Kumar *et al.*, 2004) (*S3*). Based on the ModleTest analyses results, we use the Kimura's two-parameter distance matrix (Kimura, 1980) (*S9*) and the robustness of each branch was determined by a nonparametric bootstrap test with 1000 replicates and a TBR branch swapping algorithm.

4.5.6 Estimation of population demographic parameters

Signatures of population demographic changes (e.g., bottlenecks or expansions) in domestic sheep were examined using following different approaches. First, we investigated the demographic history by comparing mismatch distributions in each haplogroup using ARLEQUIN version 3.1 ( ). In addition, the Tajima's (Fu, 1997) *(S10) D* statistic and Fu's (Tajima, 1989) (*S11*) *Fs* statistic were used to test whether the Cyt*b* data conformed to expectations of neutrality, considering that departures from neutrality could also be due to factors other than selective effects, such as a population bottleneck, a population expansion, or heterogeneity in the mutation rate. *Fs* differences were tested for significance

with a coalescent simulation program (1000 simulations), as implemented in ARLEQUIN version 3.1 (Excoffier *et al.*, 2005) (*S12*), (Table 4-5).

Growth rates of *O. orientalis*, *O. vignei* and domestic sheep were estimated with Lamarc v2.2 (Kuhner, 2006) (*S13*), using a Bayesian framework allowing migrations across taxa (with a maximum of 10000 migration events, default priors used for migration rates estimation), or without migrations. The estimation of growth rates was done with a flat prior (upper bound of 1000 and lower bound of 500), 10 initial chains (500 samples, sampling interval of 20 and burn-in period of 1000) and 2 final chains (10000 samples, sampling interval of 20 and burn-in period of 1000) (Table 4-6).

4.5.7 Estimation of the genetic diversity captured during the domestication process

A phylogenetic method was used to estimate the number of ancestral haplotypes leading to the 128 mtDNA sequences present in the contemporary sheep sample. A phylogeny of the 128 sequences was reconstructed using the software PHYML 2.4.4 (Guindon & Gascuel, 2003) (*S6*) assuming the HKY85 model of substitution. The alpha shape parameter of the gamma distribution was estimated by a maximum-likelihood method from a set of 120 wild and domestic sheep using PAML, Version 3.15 (Yang, 1997) (*S14*), under the Jukes-Cantor substitution model. We observed substantial heterogeneity in substitution rates among nucleotide sites (alpha = 0.019). To create an ultrametric tree from the phylogeny, we used the software PATHD8 (Britton *et al.*, 2007) (*S15*).

Moreover, we estimated the pairwise coalescence times. For all pairs of domestic and wild sequences we computed the genetic distances defined as the number of site differences. Genetic distances were then rescaled into coalescence times by calibrating the median distance between the A and B haplogroups at 160000 years (Pedrosa *et al.*, 2005) (*S2*) (Figure 4-4).

4.5.8 Nuclear DNA Data analysis

The nucleotide diversity was estimated for *O. vignei* (n=26), *O. orientalis* (n=11), *O. vignei* x *orientalis* (n=13) and *O. aries* (n=16), for 12 nuclear DNA loci (Table 4-*3*). First, the gametic phases were estimated for each individual using the ELB algorithm

implemented in ARLEQUIN 3.11 (Excoffier *et al.*, 2005) (*S12*), with a Dirichlet prior α value of 0.01, a ε value of 0.01, an Heterozygosity influence zone of 5 and a γ value of 0.01. The sampling interval was set to 500, the number of samples to 2000, with 100 000 burnin steps. For each locus, 11 individuals of each taxon were randomly chosen twice, and the nucleotide diversity was estimated using ARLEQUIN 3.11 (Excoffier *et al.*, 2005) (*S12*). Then, the nucleotide diversity was estimated for each taxon as the mean of the 2 replicates for the 12 loci (Figure 4-5).

4.5.9 Geographic structure of genetic diversity

The ARLEQUIN v 3.11 software (Excoffier *et al.*, 2005) (*S12*) was used for estimating the partitioning of molecular variance among regions and localities (AMOVA). The AMOVA has been performed on 98 wild individuals from the 22 populations divided into 5 geographic regions (Eastern Anatolia, Northern Zagros and Caucasus: 2, 3, 4, 5, 6, 7; Central Alborz: 10, 11, 12, 13, 23; Southern Zagros: 19, 18; Central Iranian Plateau: 20, 21, 22; Central Zagros: 8, 9, 14, 15, 16, 17; population numbers refer to Fig. 4-3 B).

4.5.10 Figures and tables

Table 4-1. Sheep and mouflon samples used in mtDNA analysis.

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Og001	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	AA000000
Og002	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og003	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og004	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og005	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og006	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og007	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og008	M08	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og009	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og010	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og011	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og012	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og013	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og014	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og015	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og016	M08	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og017	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og018	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og019	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Og020	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og021	M06	A	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og022	M36	A	*Ovis orientalis*	Iran	Ilam-Mehran	46.16	33.28	HR. Rezaei	
Og023	M36	A	*Ovis orientalis*	Iran	Ilam-Mehran	45.95	33.49	HR. Rezaei	
Og024	M36	A	*Ovis orientalis*	Iran	Ilam-Mehran	46.19	33.46	HR. Rezaei	
Og025	M36	A	*Ovis orientalis*	Iran	Ilam-Mehran	45.95	33.49	HR. Rezaei	
Og026	M36	A	*Ovis orientalis*	Iran	Ilam-Mehran	45.95	33.49	HR. Rezaei	
Og027	M36	A	*Ovis orientalis*	Iran	Ilam-Mehran	45.94	33.48	HR. Rezaei	
Og028	M36	A	*Ovis orientalis*	Iran	Ilam-Mehran	45.95	33.52	HR. Rezaei	
Og029	M36	A	*Ovis orientalis*	Iran	Ilam-Mehran	45.95	33.52	HR. Rezaei	
Og030	M36	A	*Ovis orientalis*	Iran	Ilam-Mehran	45.95	33.52	HR. Rezaei	
Og031	M36	A	*Ovis orientalis*	Iran	Ilam-Mehran	45.95	33.52	HR. Rezaei	
Og032	M06	A	*Ovis orientalis*	Turkey	Saray	44.15	38.69	A. Kence	
Og033	M06	A	*Ovis orientalis*	Turkey	Saray	44.15	38.69	A. Kence	
Og034	M28	A	*Ovis orientalis*	Iran	Azna	49.36	33.45	HR. Rezaei	
Og035	M34	A	*Ovis orientalis*	Iran	Azna	49.36	33.45	HR. Rezaei	
Og036	M28	A	*Ovis orientalis*	Iran	Azna	49.36	33.45	HR. Rezaei	
Og037	M28	A	*Ovis orientalis*	Iran	Azna	49.36	33.45	HR. Rezaei	
Og038	M28	A	*Ovis orientalis*	Iran	Azna	49.36	33.45	HR. Rezaei	
Og039	M29	A	*Ovis orientalis*	Iran	Zanjan	47.67	36.66	HR. Rezaei	
Og040	M13	B	*Ovis orientalis*	France	Corse	9.04	41.56	D. Dubray	
Og041	M15	B	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Naghash	
Og042	M35	B	*Ovis orientalis*	France	Corse	9.04	41.56	D. Dubray	
Og043	M01	E	*Ovis orientalis*	Azerbaijan	Ordubad	45.8	39.17	A. Kence	

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Og044	M01	E	*Ovis orientalis*	Azerbaijan	Ordubad	45.8	39.17	A. Kence	
Og045	M01	E	*Ovis orientalis*	Azerbaijan	Ordubad	45.8	39.17	A. Kence	
Og046	M10	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Naghash	
Og047	M10	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Naghash	
Og048	M11	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Naghash	
Og049	M12	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Naghash	
Og050	M10	E	*Ovis orientalis*	Iran	Jolfa	45.71	38.85	HR. Naghash	
Og051	M10	E	*Ovis orientalis*	Iran	Jolfa	45.71	38.85	HR. Naghash	
Og052	M10	E	*Ovis orientalis*	Iran	Jolfa	45.71	38.85	HR. Naghash	
Og053	M10	E	*Ovis orientalis*	Iran	Jolfa	45.71	38.85	HR. Naghash	
Og054	M17	E	*Ovis orientalis*	Iran	Bijar	47.51	36.07	HR. Rezaei	
Og055	M10	E	*Ovis orientalis*	Iran	Bijar	47.51	36.07	HR. Rezaei	
Og056	M01	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Rezaei	
Og057	M18	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Rezaei	
Og058	M19	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Rezaei	
Og059	M20	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Rezaei	
Og060	M17	E	*Ovis orientalis*	Iran	Bijar	47.51	36.07	HR. Rezaei	
Og061	M17	E	*Ovis orientalis*	Iran	Bijar	47.51	36.07	HR. Rezaei	
Og062	M10	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Rezaei	
Og063	M01	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Rezaei	
Og064	M10	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Rezaei	
Og065	M21	E	*Ovis orientalis*	Iran	Zanjan	47.67	36.66	HR. Rezaei	
Og066	M01	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Rezaei	
Og067	M17	E	*Ovis orientalis*	Iran	Zanjan	47.67	36.66	HR. Rezaei	

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Og068	M17	E	*Ovis orientalis*	Iran	Zanjan	47.67	36.66	HR. Rezaei	
Og069	M10	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Rezaei	
Og070	M01	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Rezaei	
Og071	M10	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Rezaei	
Og072	M01	E	*Ovis orientalis*	Iran	Marakan	45.24	38.85	HR. Rezaei	
Og073	M17	E	*Ovis orientalis*	Iran	Zanjan	47.67	36.66	HR. Rezaei	
Og074	M17	E	*Ovis orientalis*	Iran	Zanjan	47.67	36.66	HR. Rezaei	
Og075	M17	E	*Ovis orientalis*	Iran	Bijar	47.51	36.07	HR. Rezaei	
Og076	M02	Wild	*Ovis orientalis*	Iran	Sahand	54.35	27.77	HR. Naghash	
Og077	M02	Wild	*Ovis orientalis*	Iran	Sahand	46.43	37.75	HR. Naghash	
Og078	M03	Wild	*Ovis orientalis*	Iran	Sahand	46.43	37.75	HR. Naghash	
Og079	M02	Wild	*Ovis orientalis*	Iran	Sahand	46.43	37.75	HR. Naghash	
Og080	M04	Wild	*Ovis orientalis*	Iran	Bamou	52.07	29.69	HR. Naghash	
Og081	M05	Wild	*Ovis orientalis*	Iran	Bamou	52.07	29.69	HR. Naghash	
Og082	M07	Wild	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og083	M07	Wild	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og084	M07	Wild	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og085	M07	Wild	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og086	M07	Wild	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og087	M07	Wild	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og088	M09	Wild	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og089	M07	Wild	*Ovis orientalis*	Turkey	Bozdag, Konya	32.27	38.04	A. Kence	
Og090	M32	Wild	*Ovis orientalis*	Armenia	Megri	46.29	39	A. Kence	
Og091	M37	Wild	*Ovis orientalis*	Iran	Kermanshah	47.15	34.49	M. Kaboli	

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Og092	M38	Wild	*Ovis orientalis*	Iran	Shahre-Kord	51.27	31.83	M. Kaboli	
Og093	M14	Wild	*Ovis orientalis*	Iran	Lavasan	51.75	35.89	M. Mashkour	
Og094	M02	Wild	*Ovis orientalis*	Iran	Varjin	51.73	35.8	M. Mashkour	
Og095	M04	Wild	*Ovis orientalis*	Iran	Islamic Island	44.42	37.8	M. Mashkour	
Og096	M16	Wild	*Ovis orientalis*	Iran	Bijar	47.51	36.07	HR. Rezaei	
Og097	M02	Wild	*Ovis orientalis*	Iran	Bijar	47.51	36.07	HR. Rezaei	
Og098	M02	Wild	*Ovis orientalis*	Iran	Bijar	47.51	36.07	HR. Rezaei	
Og099	M02	Wild	*Ovis orientalis*	Iran	Ghazvin	49.56	36.12	HR. Rezaei	
Og100	M02	Wild	*Ovis orientalis*	Iran	Ghazvin	49.56	36.12	HR. Rezaei	
Og101	M22	Wild	*Ovis orientalis*	Iran	Ghazvin	49.56	36.12	HR. Rezaei	
Og102	M23	Wild	*Ovis orientalis*	Iran	Ghazvin	49.56	36.12	HR. Rezaei	
Og103	M22	Wild	*Ovis orientalis*	Iran	Ghazvin	49.56	36.12	HR. Rezaei	
Og104	M22	Wild	*Ovis orientalis*	Iran	Ghazvin	49.56	36.12	HR. Rezaei	
Og105	M02	Wild	*Ovis orientalis*	Iran	Zanjan	47.67	36.66	HR. Rezaei	
Og106	M33	Wild	*Ovis orientalis*	Iran	Ghazvin	49.56	36.12	HR. Rezaei	
Og107	M24	Wild	*Ovis orientalis*	Iran	Malayer	48.95	34.2	HR. Rezaei	
Og108	M24	Wild	*Ovis orientalis*	Iran	Malayer	48.95	34.2	HR. Rezaei	
Og109	M24	Wild	*Ovis orientalis*	Iran	Malayer	48.95	34.2	HR. Rezaei	
Og110	M24	Wild	*Ovis orientalis*	Iran	Malayer	48.95	34.2	HR. Rezaei	
Og111	M24	Wild	*Ovis orientalis*	Iran	Malayer	48.95	34.2	HR. Rezaei	
Og112	M24	Wild	*Ovis orientalis*	Iran	Malayer	48.95	34.2	HR. Rezaei	
Og113	M24	Wild	*Ovis orientalis*	Iran	Malayer	48.95	34.2	HR. Rezaei	
Og114	M24	Wild	*Ovis orientalis*	Iran	Malayer	48.95	34.2	HR. Rezaei	
Og115	M24	Wild	*Ovis orientalis*	Iran	Malayer	48.95	34.2	HR. Rezaei	

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Og116	M24	Wild	*Ovis orientalis*	Iran	Malayer	48.95	34.2	HR. Rezaei	
Og117	M24	Wild	*Ovis orientalis*	Iran	Malayer	48.95	34.2	HR. Rezaei	
Og118	M25	Wild	*Ovis orientalis*	Iran	Khojir	51.52	35.63	S. Naderi	
Og119	M26	Wild	*Ovis orientalis*	Iran	Gamishlou	51.21	32.85	S. Naderi	
Og120	M26	Wild	*Ovis orientalis*	Iran	Gamishlou	51.21	32.85	S. Naderi	
Og121	M26	Wild	*Ovis orientalis*	Iran	Gamishlou	51.21	32.85	S. Naderi	
Og122	M26	Wild	*Ovis orientalis*	Iran	Gamishlou	51.21	32.85	S. Naderi	
Og123	M26	Wild	*Ovis orientalis*	Iran	Gamishlou	51.21	32.85	S. Naderi	
Og124	M26	Wild	*Ovis orientalis*	Iran	Gamishlou	51.21	32.85	S. Naderi	
Og125	M26	Wild	*Ovis orientalis*	Iran	Gamishlou	51.21	32.85	S. Naderi	
Og126	M26	Wild	*Ovis orientalis*	Iran	Gamishlou	51.21	32.85	S. Naderi	
Og127	M27	Wild	*Ovis orientalis*	Iran	Malayer	48.95	34.2	HR. Rezaei	
Og128	M02	Wild	*Ovis orientalis*	Iran	Bijar	47.51	36.07	HR. Rezaei	
Og129	M25	Wild	*Ovis orientalis*	Iran	Parvar	55.5	36	S. Naderi	
Og130	M30	Wild	*Ovis orientalis*	Iran	Khabr-Kerman	56.45	28.86	S. Naderi	
Og131	M30	Wild	*Ovis orientalis*	Iran	Khabr-Kerman	56.45	28.86	S. Naderi	
Og132	M31	Wild	*Ovis orientalis*	Iran	Shahre-Babak	55.22	30.58	S. Naderi	
Og133	M31	Wild	*Ovis orientalis*	Iran	Shahre-Babak	55.22	30.58	S. Naderi	
Og134	M31	Wild	*Ovis orientalis*	Iran	Shahre-Babak	55.22	30.58	S. Naderi	
Og135	M04	Wild	*Ovis orientalis*	Iran	Bamou	52.07	29.69	S. Naderi	
Og136	M04	Wild	*Ovis orientalis*	Iran	Bamou	52.07	29.69	S. Naderi	
Og137	M04	Wild	*Ovis orientalis*	Iran	Bamou	52.07	29.69	S. Naderi	
Og138	M01	E	*Ovis orientalis*	Iran	Marakan	54.35	27.77	HR. Naghash	
Og139	M01	E	*Ovis orientalis*	Iran	Marakan	54.35	27.77	HR. Naghash	

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Og140	M39	C	*Ovis orientalis*	Iran	Kermanshah	47.15	34.49	M. Kaboli	
Ov001	U01		*Ovis vignei*	Iran	Bamou	52.07	29.69	HR. Naghash	
Ov002	U01		*Ovis vignei*	Iran	Bamou	52.07	29.69	HR. Naghash	
Ov003	U02		*Ovis vignei*	Iran	Sarigol	57.76	36.93	HR. Naghash	
Ov004	U03		*Ovis vignei*	Iran	Sarigol	57.76	36.93	HR. Naghash	
Ov005	U02		*Ovis vignei*	Iran	Sarigol	57.76	36.93	HR. Naghash	
Ov006	U04		*Ovis vignei*	Iran	Sarigol	57.76	36.93	HR. Naghash	
Ov007	U55		*Ovis vignei*	Turkmenistan	Turkmenistan	63.7	36.24	A. Kence	
Ov008	U07		*Ovis vignei*	Kazakhstan	Karaganda	71.98	49.47	P. Weinberg	
Ov009	U53		*Ovis vignei*	Iran	Roudehen	51.96	35.78	M. Mashkour	
Ov010	U54		*Ovis vignei*	Iran	?			M. Mashkour	
Ov011	U07		*Ovis vignei*	Kazakhstan	Zhabaiushkan	51.3	44.3	P. Weinberg	
Ov012	U07		*Ovis vignei*	Kazakhstan	Zhabaiushkan	51.3	44.3	P. Weinberg	
Ov013	U07		*Ovis vignei*	Kazakhstan	Zhabaiushkan	51.3	44.3	P. Weinberg	
Ov014	U38		*Ovis vignei*	Turkmenistan	Badkhyz	63.7	36.24	A. Kence	
Ov015	U39		*Ovis vignei*	Tajikistan	Karatau	60.25	42.1	AJ. Sempere	
Ov016	U40		*Ovis vignei*	Tajikistan	Karatau	60.25	42.1	AJ. Sempere	
Ov017	U40		*Ovis vignei*	Tajikistan	Karatau	60.25	42.1	AJ. Sempere	
Ov018	U07		*Ovis vignei*	Turkmenistan	Zhabaiushkan	51.3	44.3	A. Kence	
Ov019	U05		*Ovis vignei*	Iran	Neyshabour	58.55	36.63	HR. Naghash	
Ov020	U06		*Ovis vignei*	Iran	Golestan	37.43	56.14	HR. Rezaei	
Ov021	U07		*Ovis vignei*	Iran	Golestan	37.43	56.14	HR. Rezaei	
Ov022	U06		*Ovis vignei*	Iran	Golestan	37.43	56.14	HR. Rezaei	
Ov023	U08		*Ovis vignei*	Iran	Golestan	37.43	56.14	HR. Rezaei	

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Ov024	U09		*Ovis vignei*	Iran	Touran	55.83	35.77	S. Naderi	
Ov025	U09		*Ovis vignei*	Iran	Touran	55.83	35.77	S. Naderi	
Ov026	U09		*Ovis vignei*	Iran	Touran	55.83	35.77	S. Naderi	
Ov027	U09		*Ovis vignei*	Iran	Touran	55.83	35.77	S. Naderi	
Ov028	U09		*Ovis vignei*	Iran	Touran	55.83	35.77	S. Naderi	
Ov029	U10		*Ovis vignei*	Iran	Touran	55.83	35.77	S. Naderi	
Ov030	U09		*Ovis vignei*	Iran	Touran	55.83	35.77	S. Naderi	
Ov031	U11		*Ovis vignei*	Iran	Touran	55.83	35.77	S. Naderi	
Ov032	U09		*Ovis vignei*	Iran	Touran	55.83	35.77	S. Naderi	
Ov033	U09		*Ovis vignei*	Iran	Touran	55.83	35.77	S. Naderi	
Ov034	U51		*Ovis vignei*	Iran	Tandoureh	58.87	37.39	S. Naderi	
Ov035	U12		*Ovis vignei*	Iran	Tandoureh	58.87	37.39	S. Naderi	
Ov036	U12		*Ovis vignei*	Iran	Tandoureh	58.87	37.39	S. Naderi	
Ov037	U11		*Ovis vignei*	Iran	Khojir	51.52	35.63	S. Naderi	
Ov038	U11		*Ovis vignei*	Iran	Khojir	51.52	35.63	S. Naderi	
Ov039	U13		*Ovis vignei*	Iran	Salouk	57.26	37.22	S. Naderi	
Ov040	U14		*Ovis vignei*	Iran	Salouk	57.26	37.22	S. Naderi	
Ov041	U15		*Ovis vignei*	Iran	Salouk	57.26	37.22	S. Naderi	
Ov042	U16		*Ovis vignei*	Iran	Salouk	57.26	37.22	S. Naderi	
Ov043	U52		*Ovis vignei*	Iran	Nosrat abad	60.88	29.68	S. Naderi	
Ov044	U17		*Ovis vignei*	Iran	Nosrat abad	60.88	29.68	S. Naderi	
Ov045	U17		*Ovis vignei*	Iran	Nosrat abad	60.88	29.68	S. Naderi	
Ov046	U17		*Ovis vignei*	Iran	Nosrat abad	60.88	29.68	S. Naderi	
Ov047	U18		*Ovis vignei*	Iran	Nosrat abad	60.88	29.68	S. Naderi	

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Ov048	U19		*Ovis vignei*	Iran	Nosrat abad	60.88	29.68	S. Naderi	
Ov049	U20		*Ovis vignei*	Iran	Parvar	53.51	35.97	S. Naderi	
Ov050	U21		*Ovis vignei*	Iran	Parvar	53.51	35.97	S. Naderi	
Ov051	U20		*Ovis vignei*	Iran	Parvar	53.51	35.97	S. Naderi	
Ov052	U20		*Ovis vignei*	Iran	Parvar	53.51	35.97	S. Naderi	
Ov053	U09		*Ovis vignei*	Iran	Parvar	53.51	35.97	S. Naderi	
Ov054	U22		*Ovis vignei*	Iran	Parvar	53.51	35.97	S. Naderi	
Ov055	U20		*Ovis vignei*	Iran	Parvar	53.51	35.97	S. Naderi	
Ov056	U09		*Ovis vignei*	Iran	Khosh-Yeylagh	55.43	36.69	S. Naderi	
Ov057	U09		*Ovis vignei*	Iran	Khosh-Yeylagh	55.43	36.69	S. Naderi	
Ov058	U07		*Ovis vignei*	Iran	Khosh-Yeylagh	55.43	36.69	S. Naderi	
Ov059	U23		*Ovis vignei*	Iran	Kavir	52.19	34.71	S. Naderi	
Ov060	U24		*Ovis vignei*	Iran	Kavir	52.19	34.71	S. Naderi	
Ov061	U25		*Ovis vignei*	Iran	Kavir	52.19	34.71	S. Naderi	
Ov062	U23		*Ovis vignei*	Iran	Kavir	52.19	34.71	S. Naderi	
Ov063	U26		*Ovis vignei*	Iran	Kavir	52.19	34.71	S. Naderi	
Ov064	U23		*Ovis vignei*	Iran	Kavir	52.19	34.71	S. Naderi	
Ov065	U23		*Ovis vignei*	Iran	Kavir	52.19	34.71	S. Naderi	
Ov066	U23		*Ovis vignei*	Iran	Kavir	52.19	34.71	S. Naderi	
Ov067	U23		*Ovis vignei*	Iran	Kavir	52.19	34.71	S. Naderi	
Ov068	U23		*Ovis vignei*	Iran	Kavir	52.19	34.71	S. Naderi	
Ov069	U23		*Ovis vignei*	Iran	Kavir	52.19	34.71	S. Naderi	
Ov070	U23		*Ovis vignei*	Iran	Kavir	52.19	34.71	S. Naderi	
Ov071	U27		*Ovis vignei*	Iran	Khabr-Kerman	56.45	28.86	S. Naderi	

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Ov072	U27		*Ovis vignei*	Iran	Khabr-Kerman	56.45	28.86	S. Naderi	
Ov073	U27		*Ovis vignei*	Iran	Khabr-Kerman	56.45	28.86	S. Naderi	
Ov074	U27		*Ovis vignei*	Iran	Khabr-Kerman	56.45	28.86	S. Naderi	
Ov075	U28		*Ovis vignei*	Iran	Khabr-Kerman	56.45	28.86	S. Naderi	
Ov076	U29		*Ovis vignei*	Iran	Khabr-Kerman	56.45	28.86	S. Naderi	
Ov077	U30		*Ovis vignei*	Iran	Khabr-Kerman	56.45	28.86	S. Naderi	
Ov078	U31		*Ovis vignei*	Iran	Yazd	55.71	31.6	S. Naderi	
Ov079	U31		*Ovis vignei*	Iran	Yazd	55.71	31.6	S. Naderi	
Ov080	U32		*Ovis vignei*	Iran	Yazd	55.71	31.6	S. Naderi	
Ov081	U33		*Ovis vignei*	Iran	Yazd	55.71	31.6	S. Naderi	
Ov082	U34		*Ovis vignei*	Iran	Yazd	55.71	31.6	S. Naderi	
Ov083	U23		*Ovis vignei*	Iran	Shahre-Babak	55.22	30.58	S. Naderi	
Ov084	U23		*Ovis vignei*	Iran	Shahre-Babak	55.22	30.58	S. Naderi	
Ov085	U35		*Ovis vignei*	Iran	Shahre-Babak	55.22	30.58	S. Naderi	
Ov086	U36		*Ovis vignei*	Iran	Shahre-Babak	55.22	30.58	S. Naderi	
Ov087	U37		*Ovis vignei*	Iran	Bamou	52.07	29.69	S. Naderi	
Ov088	U01		*Ovis vignei*	Iran	Bamou	52.07	29.69	S. Naderi	
Ov089	U56		*Ovis vignei*	Pakistan	Nanga Parbat	74.6	35.23	AT. Virk	
Ov090	U59		*Ovis vignei*	Pakistan	Nanga Parbat	74.6	35.23	AT. Virk	
Ov091	U56		*Ovis vignei*	Pakistan	Nanga Parbat	74.6	35.23	AT. Virk	
Ov092	U60		*Ovis vignei*	Pakistan	Kharphocho	5.65	35.31	AT. Virk	
Ov093	U46		*Ovis vignei*	Pakistan	Kharphocho	5.65	35.31	AT. Virk	
Ov094	U46		*Ovis vignei*	Pakistan	Kharphocho	5.65	35.31	AT. Virk	
Ov095	U46		*Ovis vignei*	Pakistan	Kharphocho	5.65	35.31	AT. Virk	

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Ov096	U46		*Ovis vignei*	Pakistan	Kharphocho	5.65	35.31	AT. Virk	
Ov097	U41		*Ovis vignei*	Pakistan	Torghar	68.45	31.18	AT. Virk	
Ov098	U57		*Ovis vignei*	Pakistan	Torghar	68.45	31.18	AT. Virk	
Ov099	U47		*Ovis vignei*	Pakistan	Dureji	67.26	25.87	AT. Virk	
Ov100	U47		*Ovis vignei*	Pakistan	Dureji	67.26	25.87	AT. Virk	
Ov101	U42		*Ovis vignei*	Pakistan	Dureji	67.26	25.87	AT. Virk	
Ov102	U43		*Ovis vignei*	Pakistan	Dureji	67.26	25.87	AT. Virk	
Ov103	U48		*Ovis vignei*	Pakistan	Torghar	68.45	31.18	AT. Virk	
Ov104	U41		*Ovis vignei*	Pakistan	Torghar	68.45	31.18	AT. Virk	
Ov105	U49		*Ovis vignei*	Pakistan	Olia	67.2	25.31	AT. Virk	
Ov106	U44		*Ovis vignei*	Pakistan	Olia	67.2	25.31	AT. Virk	
Ov107	U45		*Ovis vignei*	Pakistan	Olia	67.2	25.31	AT. Virk	
Ov108	U58		*Ovis vignei*	Pakistan	Olia	67.2	25.31	AT. Virk	
Ov109	U58		*Ovis vignei*	Pakistan	Olia	67.2	25.31	AT. Virk	
Ov110	U50		*Ovis vignei*	Tajikistan	Pamir SE	73.3	38.1	AJ. Sempere	
Ov111	U46		*Ovis vignei*	India	Shyok	78	34	YV.Bhatnagar	
Oam01	A02		*Ovis ammon*	Uzbekistan E	Uzbekistan E	69.56	41.25	P. Weinberg	
Oam02	A03		*Ovis ammon*	Uzbekistan E	Uzbekistan E	69.56	41.25	P. Weinberg	
Oam03	A16		*Ovis ammon*	Kazakhstan	Karaganda	53.3	43.4	NI. Gidzhrati	
Oa001	D04	A	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa002	D25	A	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa003	D04	A	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa004	D04	A	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa005	D04	A	*Ovis aries*	Iran	Kermanshah			HR. Naghash	

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Oa006	D27	A	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa007	D04	A	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa008	D28	A	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa009	D29	A	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa010	D30	A	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa011	D32	A	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa012	D04	A	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa013	D04	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa014	D04	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa015	D04	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa016	D04	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa017	D40	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa018	D04	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa019	D04	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa020	D04	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa021	D04	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa022	D04	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa023	D04	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa024	D07	A	*Ovis aries*	Egypt	-				
Oa025	D07	A	*Ovis aries*	Egypt	-				
Oa026	D04	A	*Ovis aries*	Iran	Gazvin			HR. Rezaei	
Oa027	D04	A	*Ovis aries*	Iran	Azna			HR. Rezaei	
Oa028	D04	A	*Ovis aries*	Iran	Khorasan			HR. Naghash	
Oa029	D04	A	*Ovis aries*	Jordan	-				

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Oa030	D04	A	*Ovis aries*	Mongolia	-				
Oa031	D15	A	*Ovis aries*	Mongolia	-				
Oa032	D04	A	*Ovis aries*	Mongolia	-				
Oa033	D04	A	*Ovis aries*	Mongolia	-				
Oa034	D04	A	*Ovis aries*	Mongolia	-				
Oa035	D04	A	*Ovis aries*	Mongolia	-				
Oa036	D04	A	*Ovis aries*	Saudi Arabia	-				
Oa037	D01	B	*Ovis aries*	Portuguese	-				
Oa038	D02	B	*Ovis aries*	Portuguese	-				
Oa039	D03	B	*Ovis aries*	Portuguese	-				
Oa040	D22	B	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa041	D03	B	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa042	D26	B	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa043	D03	B	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa044	D36	B	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa045	D03	B	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa046	D39	B	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa047	D05	B	*Ovis aries*	Azerbaijan	-				
Oa048	D06	B	*Ovis aries*	Azerbaijan	-				
Oa049	D03	B	*Ovis aries*	India	-				
Oa050	D03	B	*Ovis aries*	Iran	Kerman			S. Naderi	
Oa051	D08	B	*Ovis aries*	Iran	Gazvin			HR. Rezaei	
Oa052	D03	B	*Ovis aries*	Iran	Marakan			HR. Rezaei	
Oa053	D09	B	*Ovis aries*	Iran	Ghorveh			HR. Rezaei	

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Oa054	D10	B	*Ovis aries*	Iran	Mehran			HR. Rezaei	
Oa055	D03	B	*Ovis aries*	Iran	Mehran			HR. Rezaei	
Oa056	D11	B	*Ovis aries*	Iran	Marakan			HR. Rezaei	
Oa057	D20	B	*Ovis aries*	Libya	-				
Oa058	D03	B	*Ovis aries*	Mongolia	-				
Oa059	D17	B	*Ovis aries*	Saudi Arabia	-				
Oa060	D03	B	*Ovis aries*	Saudi Arabia	-				
Oa061	D03	B	*Ovis aries*	Sudan	-				
Oa062	D21	C	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa063	D23	C	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa064	D19	C	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa065	D19	C	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa066	D31	C	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa067	D33	C	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa068	D34	C	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa069	D41	C	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa070	D37	C	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa071	D13	C	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa072	D13	C	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa073	D41	C	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa074	D41	C	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa075	D12	C	*Ovis aries*	Iran	Khorasan			HR. Naghash	
Oa076	D13	C	*Ovis aries*	Kazakhstan	-				
Oa077	D14	C	*Ovis aries*	Kazakhstan	-				

Sample	*Haplotype*	*Haplogroup*	*Species*	*Country*	*Place of Sampling*	*Longitude*	*Latitude*	*Collector*	*Accession No.*
Oa078	D18	C	*Ovis aries*	Kirgizstan	-				
Oa079	D19	C	*Ovis aries*	Kirgizstan	-				
Oa080	D24	E	*Ovis aries*	Iran	Kermanshah			HR. Naghash	
Oa081	D16	E	*Ovis aries*	Uzbekistan	-				
Oa082	D35	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	
Oa083	D38	A	*Ovis aries*	Iran	Sanandadj			HR. Naghash	

Table 4-2. Wild and domestic *Ovis* Cyt*b* sequences obtained from GenBank.

Accession No	*Taxon*	*Accession No*	*Taxon*
AJ867268 [a]	*Ovis ammon*	AJ867266 [a]	*Ovis ammon*
AF242349 [z]	*Ovis ammon ammon*	AJ867260 [a]	*Ovis ammon*
AF242350 [z]	*Ovis ammon darwini*	AJ867257 [a]	*Ovis ammon*
AF034727 [b]	*Ovis ammon darwini*	AJ867275 [a]	*Ovis ammon*
AJ867276 [a]	*Ovis ammon*	AJ867261 [a]	*Ovis vignei*
AJ867272 [a]	*Ovis ammon*	D84203 [z]	*Ovis orientalis musimon*
AJ867269 [a]	*Ovis ammon*	AJ867267 [a]	*Ovis ammon*
DQ097408 [c]	*Ovis aries*	DQ903212 [d]	*Ovis aries*
DQ097407 [c]	*Ovis aries*	DQ903221 [d]	*Ovis aries*
DQ097415 [c]	*Ovis aries*	DQ903226 [d]	*Ovis aries*
DQ097423 [c]	*Ovis aries*	DQ903210 [d]	*Ovis aries*
DQ097427 [c]	*Ovis aries*	DQ097416 [c]	*Ovis aries*
DQ097413 [c]	*Ovis aries*	DQ097409 [c]	*Ovis aries*
DQ097412 [c]	*Ovis aries*	DQ097430 [c]	*Ovis aries*
DQ097410 [c]	*Ovis aries*	DQ097425 [c]	*Ovis aries*
DQ097414 [c]	*Ovis aries*	DQ097424 [c]	*Ovis aries*
DQ097411 [c]	*Ovis aries*	DQ097426 [c]	*Ovis aries*
DQ097422 [c]	*Ovis aries*	DQ097429 [c]	*Ovis aries*
DQ097421 [c]	*Ovis aries*	DQ097428 [c]	*Ovis aries*
DQ097420 [c]	*Ovis aries*	DQ903208 [d]	*Ovis aries*
DQ097419 [c]	*Ovis aries*	DQ903209 [d]	*Ovis aries*
DQ097418 [c]	*Ovis aries*	DQ903211 [d]	*Ovis aries*
DQ097417 [c]	*Ovis aries*	DQ903213 [d]	*Ovis aries*
DQ903217 [d]	*Ovis aries*	DQ903214 [d]	*Ovis aries*
DQ903218 [d]	*Ovis aries*	DQ903215 [d]	*Ovis aries*
DQ903219 [d]	*Ovis aries*	DQ903216 [d]	*Ovis aries*
DQ903220 [d]	*Ovis aries*	DQ903225 [d]	*Ovis aries*
DQ903222 [d]	*Ovis aries*	DQ903227 [d]	*Ovis aries*
DQ903223 [d]	*Ovis aries*	DQ903224 [d]	*Ovis aries*
DQ097423 [c]	*Ovis aries*	DQ097427 [c]	*Ovis aries*

[a] (Bunch *et al.*, 2006) (S16), [b] (Hassanin *et al.*, 1998) (S17), [c] (Pedrosa *et al.*, 2005) (S2), [d] (Wang *et al.*, 2006) (S18) and [z] Unpublished.

Table 4-3. *Ovis* samples used for nuclear DNA analysis.

Sample	Species	Country	Place of Sampling
OarMH1	*O. aries*	Mongolia	
OarMH2	*O. aries*	Mongolia	
OarMH3	*O. aries*	Mongolia	
OarMH4	*O. aries*	Mongolia	
OarMH5	*O. aries*	Mongolia	
OarMH6	*O. aries*	Mongolia	
OarMH7	*O. aries*	Mongolia	
OarMH8	*O. aries*	Mongolia	
902MB	*O. aries*	Portugal	
903MB	*O. aries*	Portugal	
904MB	*O. aries*	Portugal	
905MB	*O. aries*	Portugal	
906MB	*O. aries*	Portugal	
907MB	*O. aries*	Portugal	
908MB	*O. aries*	Portugal	
909MB	*O. aries*	Portugal	
OoscI2	*O. orientalis*	Iran	Bamou
OoI101	*O. vignei*	Iran	Rudehen
OoI301	*O .orientalis*	Iran	Lavasan
OoI801	*O. vignei*	Iran	?
OoI901	*O .orientalis*	Iran	Damavand
OoI1001	*O. vignei*	Iran	Rudehen
OoI1101	*O .orientalis*	Iran	Lavasan
OoI1301	*O. orientalis*	Iran	Varjin
OvKaz1	*O. vignei*	Kazakhstan	Egendebukak
OvKaz2	*O. ammon*	Kazakhstan	Karaganda
OvKaz3	*O. vignei*	Kazakhstan	Zhabaiushkan
OvKaz4	*O. vignei*	Kazakhstan	Zhabaiushkan
OgArm1	*O. orientalis*	Armenia	Megri
OgArm2	*O .orientalis*	Armenia	Zangezur
OgArm3	*O .orientalis*	Armenia	Zangezur
OgTk1	*O .orientalis*	Turkey	Bozdag
OgTk2	*O. orientalis*	Turkey	Saray
OgTk3	*O. orientalis*	Turkey	Saray
OgIr01	*O. orientalis*	Iran	Bijar
OgIr02	*O. orientalis*	Iran	Marakan
OgIr03	*O. orientalis*	Iran	Gazvin

OgIr04	*O. orientalis*	Iran	Malayer
OvIr05	*O. vignei*	Iran	Tandoureh
OvIr06	*O. vignei*	Iran	Tandoureh
OvIr07	*O. vignei*	Iran	Khojir
OvIr08	*O. vignei*	Iran	Khojir
OgIr09	*O. orientalis*	Iran	Azna
OgIr10	*O. orientalis*	Iran	Azna
OvIr11	*O. vignei*	Iran	Nosrat abad
OvIr12	*O. vignei*	Iran	Nosrat abad
OvIr13	*O. vignei*	Iran	Parvar
OvIr14	*O. vignei*	Iran	Parvar
OvIr15	*O. vignei*	Iran	Khosh-Yeylagh
OvIr16	*O. vignei*	Iran	Kavir
OvIr17	*O. vignei*	Iran	Kavir
OvTkm1	*O. vignei*	Turkmenistan	Zhabaiushkan
OvTkm2	*O. vignei*	Turkmenistan	Khugitang mts
OvTkm3	*O. vignei*	Turkmenistan	Badkhyz
OvTkm4	*O. vignei*	Turkmenistan	Zhabaiushkan
OvTaj1	*O. vignei*	Tajikistan	Karatau
OvTaj2	*O. vignei*	Tajikistan	Karatau
OvTaj3	*O. vignei*	Tajikistan	Karatau
OvTaj4	*O. vignei*	Tajikistan	Pamir SE
OvPk01	*O. vignei*	Pakistan	Kharphocho
OvPk02	*O. vignei*	Pakistan	Kharphocho
OvPk03	*O. vignei*	Pakistan	Torghar
OvPk04	*O. vignei*	Pakistan	Dureji
OvPk05	*O. vignei*	Pakistan	Dureji
OvPk06	*O. vignei*	Pakistan	Dureji
OvPk07	*O. vignei*	Pakistan	Torghar
OvPk08	*O. vignei*	Pakistan	Torghar
OvPk09	*O. vignei*	Pakistan	Olia
OvPk10	*O. vignei*	Pakistan	Olia
OvPk11	*O. vignei*	Pakistan	Olia
OvaUz1	*O. ammon*	Uzbekistan	Uzbekistan E
OvaUz2	*O. ammon*	Uzbekistan	Uzbekistan E

Table4-4. Primers used for amplifying mtDNA and nuclear DNA.

Lcus	Primer	Sequence of the Primer (5'-3')	Length of fragment (Base Pair)	TM	TM
Cytochrome *b* (Part 1)	CYTB_F	CCCCACAAAACCTATCACAAA	741	52	55
	CYTB_IN_R	CCTGTTTCGTGGAGGAAGAG		62	
Cytochrome *b* (Part 2)	CYTB_IN_F	ACCTCCTTTCAGCAATTCCA	765	58	60
	CYTB_R	AGGGAGGTTGGTTGTTCTCC		62	
Kappa casein	Kcas-X4F	AGAAATAATACCATTCTGCAT	498	43	50
	Kcas-X4R	TTGTCTTCTTTGATGTCTCCTTAGAG		52	
Similar to Hypothetical Protein	HSPC148-F	GGGATGATGACGTTGTTTTC	293	58	58
	HSPC148-R	GGGTTAAACCAATTCCCAAG		58	
Interleukin 16	IL16-F	CCAGGCAAGCTGTGATCGT	423	60	58
	IL16-R	GAAGATCCTGTTAACTGTCAGAGG		51	
Growth differentiation factor 9B	GDF9BF	ACTCCGCTTCGTGTGTCAGC	483	64	58
	GDF9BR	TACTCCCATTTGCCTCAATC		58	
Zona Pellucida 3	ZP3A-X3F	TGCCATTCAGGACCACAGT	279	58	58
	ZP3A-X4R	GGAAGTCCACGATGGTGTG		60	
Zona Pellucida 3	ZP3A-X4F	GAGAAGATGACGCCCACCT	319	60	58
	ZP3A-X5RA	CATTAGCAAAACGGAACACATC		52	
Zona Pellucida 2	ZP2-X8F	CCATCTCTACATGGTGCCTCT	310	51	51
	ZP2-X9R	TTGTTTTGAGGAGAGTTTTGCT		50	
Toll-like receptor 2	TLR2-X2Fa	GACCTGCAGAGGTGTGTGAA	471	62	58
	TLR2-X2Ra	TGAAAAATGGAAAGTGTGCAA		51	
Keratin Associated Protein 1.3	KAP1-3F	GGGTGGAACAAGCAGACCAAACTC	584	60	58
	KAP1-3R	AAGTTTGTTGGGACTGTACACTGGC		57	
Capra hircus microsatellite	U80588-Fb	AGTATCTTTTCTTGCATTTGTTTCC	241	52	58
	U80588-Rb	CACAGGGGTTTCTGGTTGG		60	
Interleukin 4	IL4-X1R	TCACATTGTCAGTGCAAATAGAG	436	58	55
	IL4-X1F	TTTGGGGCAGCAAAGACGT		50	
Toll-like receptor 4	TLR4X4Fb	TTCAAGGGTTGCTGTTCTCA	379	58	55
	TLR4X4Rb	CAGCACCTGAAGGCTAGAGAG		51	

Table 4-5. The results of Fu test.

	Statistics	A	B	C & E	Mean	s.d.
Tajima's D test						
	Sample size	52	45	31	42.6667	8.7305
	S	29	40	30	33	4.9666
	Pi	1.2986	2.2677	3.3613	2.3092	0.8426
	Tajima's D	-2.6309	-2.5983	-1.9843	-2.4045	0.2974
	Tajima's D p-value	0.0000	0.0000	0.0080	0.0027	0.0038
Fu's FS test						
	No. of alleles(unchecked)	52	45	31	42.6667	8.7305
	Theta pi	1.2986	2.2677	3.3613	2.3092	0.8426
	Exp. no. of alleles	5.3728	7.3991	8.2891	7.0203	1.2203
	FS	-28.3883	-26.8621	-26.1181	-27.1228	0.9449
	FS p-value	0.0000	0.0000	0.0000	0.0000	0.0000

Table 4-6. Results of lamarc.

Taxa	Growth rate	95% percentile
Domestics	991.2544	859.3855-1001.613
	987.6652	866.3764-1002.177
	989.7268	868.5301-1002.274
	988.7643	881.6321-1000.251
O. orientalis	839.6221	391.7040-996.4198
	574.3359	314.1113-938.0520
	924.2447	409.4828-1001.935
	566.1506	249.0274-891.6057
O. vignei	843.5658	484.8535-991.9876
	826.2423	549.0939-1000.490
	725.7407	540.4904-1004.101
	963.9504	476.3987- 1008.922
Domestics	989.4532	868.5209-1001.416
	992.0691	867.9568-1004.754
	990.2076	851.1182- 1003.150
	992.3093	874.856- 1000.957
O. orientalis close-to-domestics	914.2827	-125.5870-1018.213
	795.3570	-82.91215-997.2565
	823.3480	-256.3642- 1017.871
	853.8762	83.16194- 1000.894
O. orientalis non close-to-domestics	915.5454	405.6693-994.4154
	654.9602	305.4288- 983.2233
	921.7286	280.3691-1008.754
	931.8407	396.7605- 1004.979
O. vignei	744.9049	545.3356-988.6498
	710.6284	470.7356- 982.3060
	701.8297	500.1019- 969.0823
	705.6038	398.7940- 990.0955

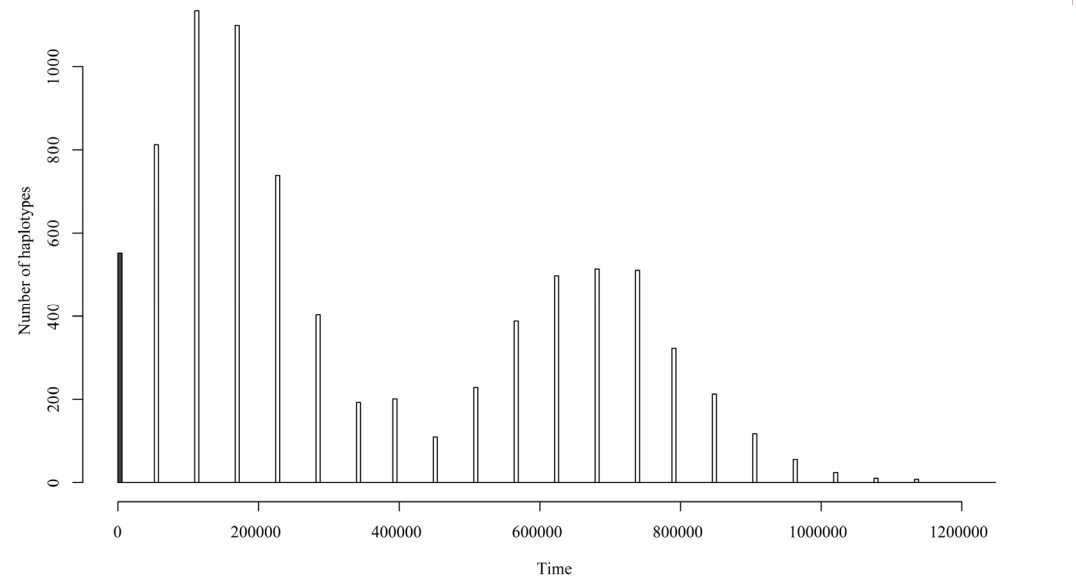

Figure 4-4. Pairwise coalescence times of sheep (*Ovis aries*) mtDNA haplotypes. Genetic distances are computed as the number of differences between pairs of sequences and are then rescaled in time by using 160,000 years for the divergence time between A and B haplogroups. The shaded part of the histogram corresponds to the pairs of sequences that coalesced more recently than the domestication.

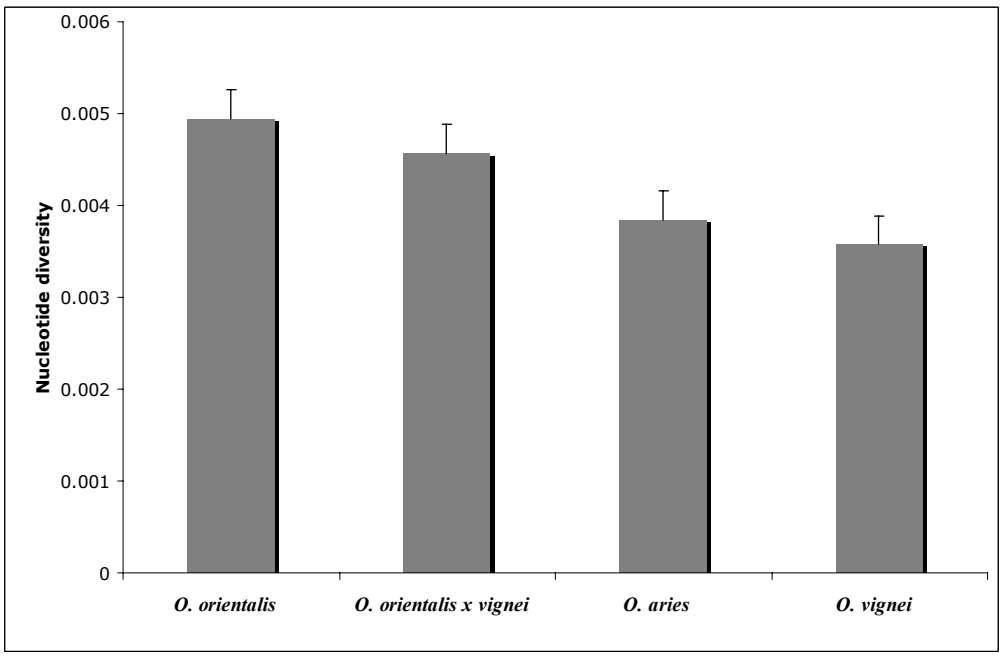

Figure 4-5. Genetic diversity of the domestic sheep compared to those of *Ovis orientalis*, *O. vignei* and *O. orientalis* x *vignei*.

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Chapter 5

Are Cattle, Sheep, and Goats Endangered Species?

5. Are Cattle, Sheep, and Goats Endangered Species?

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Running title: Are cattle, sheep, and goats endangered species?

5.1 Abstract

For about 10000 years, farmers have been managing cattle, sheep, and goats in a sustainable way, leading to animals that are well adapted to the local conditions. About two hundreds years ago, the situation started to change dramatically, with the rise of the concept of breed. All animals from the same breed began to be selected for the same phenotypic characteristics, and reproduction among breeds was seriously reduced. This corresponded to a strong fragmentation of the initial populations. A few decades ago, the selection pressures were increased again in order to further improve productivity, without enough emphasis on the preservation of the overall genetic diversity. The efficiency of modern selection methods successfully increased the production, but with a dramatic loss of genetic variability. Many industrial breeds now suffer from inbreeding, with effective population sizes falling below 50. With the development of these industrial breeds came economic pressure on farmers to abandon their traditional breeds, and many of these have recently become extinct as a result. This means that genetic resources in cattle, sheep, and goats are highly endangered, particularly in developed countries. It is therefore important to take measures that promote a sustainable management of these genetic resources, first by *in situ* preservation of endangered breeds, second by using selection programs to restore the genetic diversity of industrial breeds, and finally by protecting the wild relatives that might provide useful genetic resources.

5.2 Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), the population sizes of domestic cows, sheep, and goats, are about 1,400, 1,100, and 700 million, respectively (Scherf, 2000) (Table 5-1). Over the past 15 years, about 300 of 6000 breeds of farm animals identified by the FAO have become extinct. Furthermore, 1350 breeds of domestic animals currently face extinction in the near future (Scherf, 2000). This trend of loss of cattle, sheep, and goat breeds appears particularly strong in Europe (Table 5-1), possibly because it remains poorly documented in developing countries. At the worldwide level, 17% of cattle and 14% of sheep breeds have already been lost (Scherf, 2000).

The International Union for the Conservation of Nature and Natural Resources (IUCN) regards a species as critically endangered, endangered, or vulnerable when its effective population size falls below 50, 250, or 1000, respectively (IUCN, 2000). The rule-of-thumb in conservation biology considers that the effective population size should not be lower than 50 to avoid extinction in the short-term, and not lower than 500 to avoid extinction in the long term (Franklin, 1980).

Thus, it seems irrelevant to consider these three domestic species as endangered, considering their numbers that in the case of random mating result in effective population sizes way above the critical thresholds. However, such conclusions based purely on the number of individuals are often overly simplistic. After a brief presentation of the domestication history of these three species, we will separately consider the cases of highly productive breeds and of local breeds with low population sizes. We will examine the potential threats that cattle, sheep, and goats might suffer from, with emphasis on the current management, particularly in developed countries. These three domestic species are divided into many breeds (Table 5-1), and each breed can be considered as an independent genetic unit, as crosses are not usually employed for reproduction in developed countries. Is the current management of breeds of high commercial value sustainable? What is the impact of managing these breeds separately, of the extensive use of artificial insemination, and of increasing the selection pressure for higher production? What are the optimal management guidelines for a sustainable use of genetic resources in cattle, sheep, and goats?

From a conservation biology point of view, our goal is also to show the possible parallel between domestic and wild species. Do domestic and wild species suffer from the same threats? Should the same concepts be used for managing wild and domestic animals?

5.3 Wild Ancestors and the Domestication Process

Beside the wild ancestor when it still exists, the breeds to be used as genetic resources (i.e. the breeds with the highest genetic diversity) are expected to be found close to the domestication centres. As a consequence, precise knowledge of wild ancestors, of domestication centres, and of colonization routes is of prime importance for tracking genetic resources.

Information about cattle, sheep, and goat domestication comes from archaeological evidence, mostly from osteometry and morphometry, but also from genetic data (Vigne *et al.*, 2005b). Up to now, genetic studies on domestication mainly concerned the analysis of mitochondrial DNA (mtDNA) polymorphisms, either in the domestic species itself, or by comparing the domestic species with its wild ancestor.

5.3.1 Cattle

It is now widely recognized that the wild ancestor of all domesticated cattle was the auroch (*Bos primigenius*) (Zeuner, 1963). The aurochs are now extinct. For domestic cattle, the common usage accepts two taxa (*Bos taurus* and *B. indicus*) that fully interbreed. *B. indicus* differs from *B. taurus* by the presence of a prominent hump. The mtDNA polymorphism reflects this dichotomy (Figire 5-1), but the reality is much more complex due to extensive hybridization among these two cattle haplogroups in Africa (Bradley *et al.*, 1996).

The presence of two mtDNA haplogroups is interpreted as an indication of two main domestication events, one in the Fertile Crescent leading to *B. taurus*, and one in the Indian sub-continent leading to *B. indicus* (Bradley *et al.*, 1996; Bradley & Magee, 2006; Loftus *et al.*, 1994). Eighty four percent of the mitochondrial variation is partitioned among Europe, Asia, and Africa (Bradley *et al.*, 1996). The earliest archaeological evidence of cattle domestication dates from 8800 to 8300 BC (calibrated) in the Fertile Crescent (Helmer *et al.*, 2005).

5.3.2 Sheep

Archaeological evidence indicates that domestic sheep, *Ovis aries*, were also domesticated in the Fertile Crescent, *circa* 8500 BC (calibrated) (Peters *et al.*, 2005). However, their wild ancestors have not yet been identified with certainty, as no extensive genetic studies have been carried out on the putative ancestors. The wild candidates are *Ovis orientalis* (the Asiatic mouflon), *O. vignei* (the urial), and *O. ammon* (the argali), with a preference for *O. orientalis*, which shows the same chromosomal numbers as the domestic species (Bruford & Townsend, 2006).

To date, four main mitochondrial DNA haplogroups have been found in domestic sheep, indicating multiple maternal origins (Figire 5-1), and 35% of the mtDNA variation is partitioned among continents ((Townsend, 2000), cited by (Bruford *et al.*, 2003).

5.3.3 Goat

Goat domestication is very well documented. The first archaeological evidence traces back as far as 8500-7900 BC (calibrated) in the Zagros mountains (Fertile Crescent) (Fernández *et al.*, 2005; Luikart *et al.*, 2006), and the wild ancestor is the bezoar, *Capra aegagrus* (Luikart *et al.*, 2001).

The main characteristic of goat mtDNA polymorphism is its large haplotypic variation and its weak intercontinental phylogeographic structure, with only 10% partitioned among continents, suggesting high historical gene flow among continents (Fernandez *et al.*, 2006). A recent ancient DNA study suggested that high gene flow already occurred during the Neolithic expansion into Europe (Kumar *et al.*, 2004). Up to now, five different mtDNA haplogroups have been found (Figire 5-1), indicating multiple maternal origins, as in sheep and cattle.

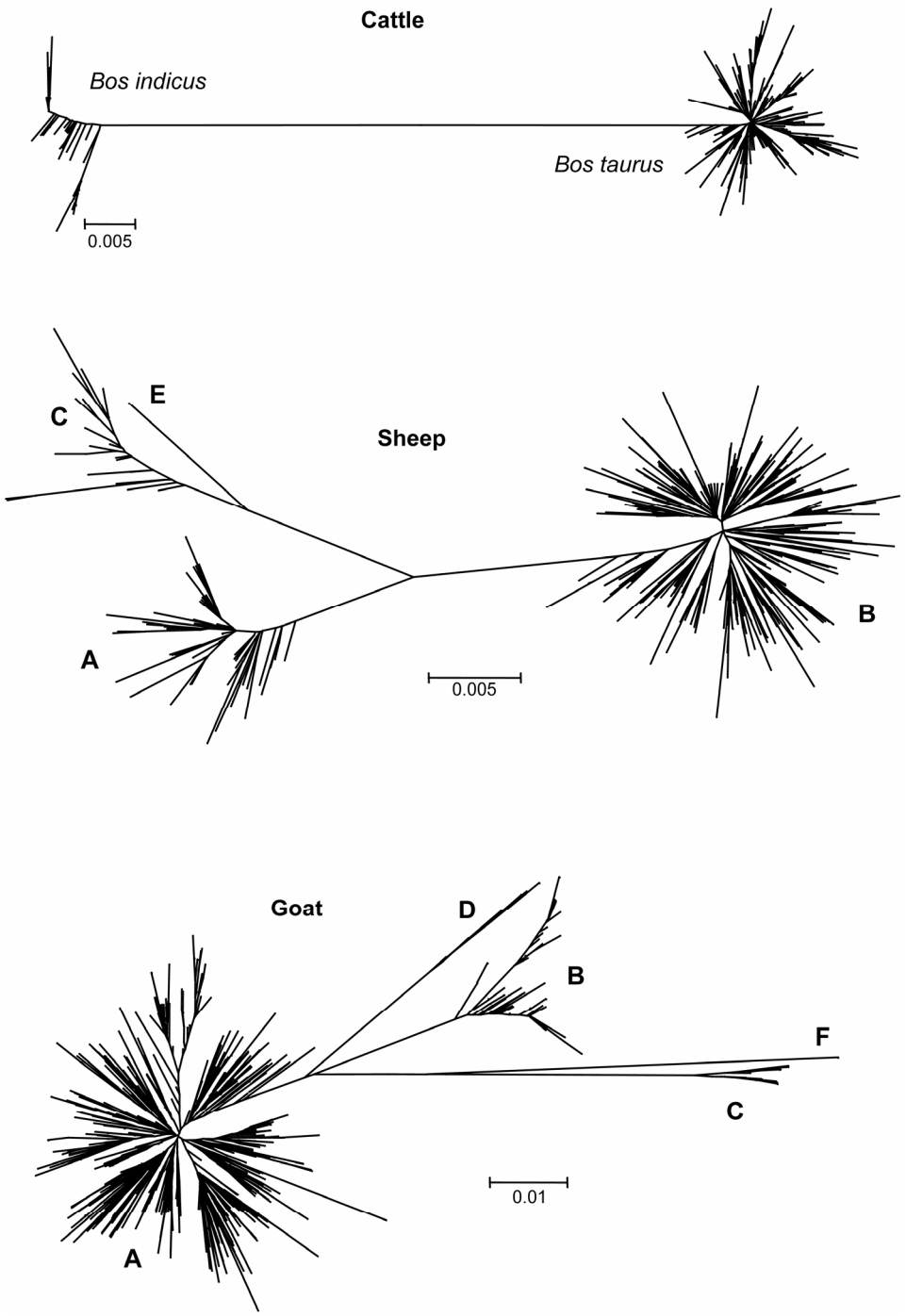

Figure 5-1 Unrooted neighbor-joining trees showing the mtDNA polymorphism of cattle, sheep, and goats.The phylogenetic analyses were conducted using MEGA version 3.1, (Kumar *et al.*, 2004), with control region sequences. A total of 744 sequences from (Bradley *et al.*, 1996; Loftus *et al.*, 1994; Troy *et al.*, 2001) were used for cattle. A total of 640 sequences from (Guo *et al.*, 2005; Hiendleder *et al.*, 1998; Meadows *et al.*, 2005; Pedrosa *et al.*, 2005; Tapio *et al.*, 2006; Wood & Phua, 1996) were used for sheep. A total of 1813 sequences from (Azor *et al.*, 2005; Bradley & Magee, 2006; Chen *et al.*, 2005; Joshi *et al.*, 2004; Li *et al.*, 2006; Liu *et al.*, 2007; Luikart *et al.*, 2001; Odahara *et al.*, 2006; Pereira *et al.*, 2005; Sardina *et al.*, 2006; Sultana *et al.*, 2003) were used for goats. The letters A, B, C, etc. in the trees for sheep and goats represent the different mtDNA haplogroups described in the literature.

5.3.4 Dispersal from the Domestication Centers

During the 3000-4000 years following the initial domestication events in the Fertile Crescent, agriculture spread over Europe, Africa, and Asia. Archaeological evidence showed that two main colonization routes took place in Europe (Figire 5-2): the Mediterranean route and the Danubian route. A decrease of genetic diversity likely occurred during this colonization process in Europe. This has been demonstrated for cattle mtDNA, for which populations in Western Europe exhibit lower polymorphism than those in the Near East (Anderung *et al.*, 2005; Beja-Pereira *et al.*, 2006; Cymbron *et al.*, 1999; Miretti *et al.*, 2004). A number of secondary livestock migrations accompanied human migrations in more recent historical times and contributed to the shaping of local gene pools. For instance an introgression of the African gene pool is observed in Iberian cattle breeds (Pellecchia *et al.*, 2007), possibly linked to the Moorish occupation or to even earlier events. Also, a surprisingly high level of mtDNA variation and close genetic relationship was discovered between Tuscan cattle breeds and Near Eastern breeds. This pattern might be linked either to the sailing and docking in Tuscany of Middle Eastern people in the late Bronze Age and to the onset of the Etruscan civilization in Central Italy (Beja-Pereira *et al.*, 2006), or to an introgression from local aurochs (Zeder *et al.*, 2006).

Overall, the level of mtDNA polymorphism in cattle, sheep, and goats (Figire 5-1) is high, and contains evidence of multiple maternal origins. Such multiple origins correspond either to several domestication events in different locations and/or at different periods, or to the capture of several mtDNA haplotypes during a single domestication event. Furthermore, nuclear DNA polymorphism seems high (see e.g. Maudet *et al.* 2002), comparable to what is found in wild species. During crop domestication, many species went through a strong bottleneck (see references in (Epstein & Mason, 1984)), but this is clearly not the case for livestock. All the current evidence suggests that cattle, sheep, and goats have very large gene pools on which human induced-selection was acting to produce the very large diversity of breeds we observe today.

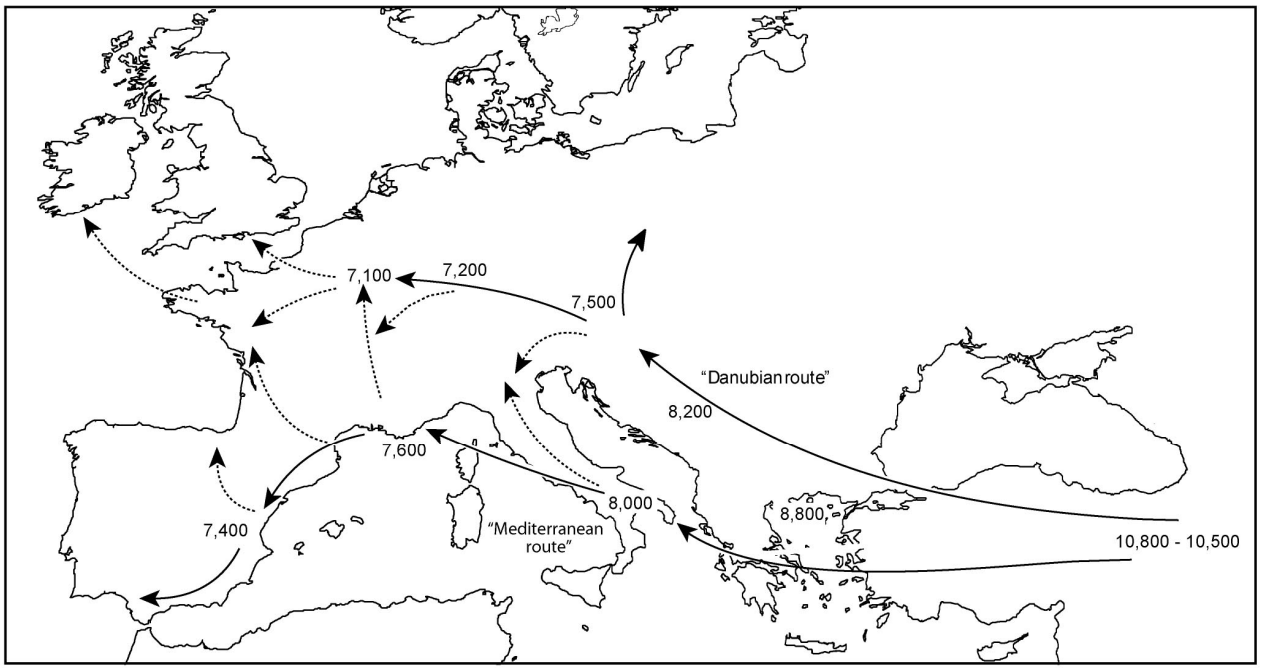

Figure 5.2 The two main initial advancements of the Neolithic culture into Europe (from Fernàndez *et al.* 2006).The dates on the map are calibrated radiocarbon date-derived BP, and correspond to the arrival of agriculture in the corresponding region.

5.4 The Threats on Highly Productive Breeds

5.4.1 Fragmentation into Discrete Breeds

About 10000 years ago, farmers started controlling the reproduction of their animals, by favouring the reproduction of animals with preferred phenotypes, and using males either from their own farm, or from another farm located in the same area. As a consequence, farm animals slowly became locally adapted. About two hundred years ago, the situation started to dramatically change. Stronger selection pressures were applied to local populations followed by standardization of the desired conformation and performance. The first cattle herd book was published in Britain in 1822 (Vishwanath, 2003). This led to the concept of breed. All animals from the same breed began to exhibit the same phenotypic characteristics, and reproduction among different phenotypes (i.e. among different breeds) was seriously reduced. A few decades ago, selection pressures were increased again in order to further improve productivity. To summarize, farm animals underwent relatively low selection pressures during about 98% of their common history

with humans, and later their populations were suddenly fragmented into many well-defined breeds, with high selection constraints.

5.4.2 Effects of Artificial Insemination and other Reproductive Technologies

Artificial insemination offers the possibility of easily obtaining thousands of progeny from a single sire, permitting the dissemination of valuable genes (Boichard *et al.*, 1996). It is widely used in cattle, particularly in dairy farms, and is the main method of reproduction in many breeds in the developed world, while in sheep and goats it is limited to a few highly productive breeds. This has greatly sped up the rate of genetic change of livestock populations by increasing the selection pressure and the reliability of sire breeding values, estimated from the performance of a large number of relatives. "Improved" germplasm has flooded almost every market, displacing locally adapted populations and inducing the loss of many genetic variants.

The effect of artificial insemination on effective population size is sometimes striking (Table 5-2). For example, *Ne* is as low as 46 in French Holstein, a breed that counts 2.5 million animals across France (Nomura *et al.*, 2001). An even more extreme result was found in Japan, where the Japanese Black cattle had a *Ne* of 17.2 in between 1993 and 1997, despite a census size of 0.53 million reproductive cows (Maiwashe & Blackburn, 2004; Tapio *et al.*, 2005). A reduction in effective population size has also been documented in sheep (Maudet & Taberlet, 2002), and is probably occurring in goat breeds where artificial insemination has been implemented. Surprisingly, rather high levels of genetic diversity at the nuclear DNA level still appear to exist in the Holstein cattle population, with observed heterozygosity above 0.6 (0.67 in (Maudet *et al.*, 2002a); 0.61 in (Vallejo *et al.*, 2003)). Such a level of heterozygosity is probably highly overestimated due to an ascertainment bias produced by non-random sampling of the genetic markers used (Rogers & Jorde, 1996). The microsatellites used were selected among a large set of potential markers, with the goal of maximizing the level of polymorphism and/or heterozygosity. They are probably mainly located in chromosomal regions that have not been under selection. The markers that are either monomorphic or have a low level of polymorphism are simply ignored and are usually not reported in the literature.

Another problem could be the oversimplification of the models for estimating genetic values, only involving simple linear models that do not consider interactions between factors. As a consequence, they do not take into account dominance and epistasis

effects, thus overestimating the genetic value of heterozygotes which are consequently more likely to be selected for reproduction (Cappuccio *et al.*, 2003). Nevertheless, attention needs to be paid to the maintenance of sufficient within breed genetic diversity, to preserve populations from falling into the extinction vortex (Soulé & Mills, 1998) and guarantee the long-term sustainable exploitation of livestock.

Inbreeding has always been avoided by breeders. Traditional practices included the exchange of parents among herds, culling of parents when daughters became sexually mature or confinement in breeding groups with mating with alternate males. Artificial insemination made these practices unfeasible. Most semen doses in the market arise from related bulls and this information is not easily available to single breeders, so unwanted inbreeding is likely to occur; semen doses are available for a long time after a bull is dead, making an insemination with its descendants more likely; most pedigrees do not go back more than three generations and therefore grouping females according to the common recent ancestry will not prevent mating with a relative male.

Artificial selection always reduces the number of genetic variants passed on to the following generation and with time it leads to the fixation of the desired alleles. The high level of linkage disequilibrium observed in livestock species (Farnir *et al.*, 2000; Khatkar *et al.*, 2006) may favour additional fixation of rather large chromosome regions flanking genes under intense selection, by the hitch-hiking process (Maynard Smith & Haigh, 1974). Also, random sampling of a few parents as with artificial insemination may lead to fixation of genes unlinked to the gene under selection by chance. The selection schemes currently employed in cattle may make the fixation process particularly rapid. In practice, young bulls enter the progeny test scheme on a pedigree index computed by the BLUP-Animal Model (Henderson *et al.*, 1959). The index is built by using the genetic value of relatives weighted by their relatedness with the candidate bull. Therefore, young bulls belonging to a family with good records are more likely to be included in the progeny test program. In this way the genetic pool of the group of parents of the next generation is dramatically reduced, even before genetic evaluation. After the progeny test, genetic indexes are computed with the same statistical procedure. Although the contribution of relatives has less weight here since records of the candidate (or that of its daughters) are considered as well, bulls in a "good" family still tend to have better indexes. Consequently, allelic variants are lost in an exponential way by the combination of selection and of preferential choice across families.

Increasing the selection pressure by the use of a lower number of sires per generation results in the reduction of the effective population size (*Ne*, see above) and the increase of inbreeding, which has short-term negative effects on productivity, particularly on reproductive traits. Hence it is not surprising that in highly selected dairy cattle breeds, a continuous and alarming decrease in fertility has been observed in the last 10 to 20 years in countries in which fertility traits are not sufficiently taken into consideration in the selection objectives (e.g. (Lucy, 2001)). In addition, inbreeding can promote the emergence of new hereditary diseases, such as the "complex vertebral malformation" (Malher *et al.*, 2006), which have strong detrimental economic effects on farms.

Artificial insemination has also dramatically changed the sex ratio, particularly in dairy cattle breeding, since its introduction into current practice in the past century. The ratio has declined from 1 to 10 – 30 males/females to 1 to several hundred (Rabasa, 1950). A very low sex ratio leads to a strong reduction of the effective population size, and thus to inbreeding.

5.5 The Threats on Local Breeds with Low Population Sizes

5.5.1 Socio-Economic Context

The major threats to livestock genetic diversity result from systemic, regional and global economic forces and changing agricultural practices. Intensification of production systems, including the wider availability of vaccines and therapeutics against endemic diseases, promotes the use of higher-production, less well-adapted genotypes. These facts, combined with the progressive abandonment of agriculture in marginal areas and the success of industrial breeding, have led farmers to partially or completely abandon a number of autochthonous breeds. The lack of application of methods for estimating the real economic value of these breeds, beside the value of meat, milk, and wool production, is also partially responsible for this trend (Roosen *et al.*, 2005). As a consequence, many locally adapted populations have been greatly reduced, posing the new problems of genetic drift and inbreeding to their ranchers.

Table 5-1. Population sizes, current number of breeds, number of extinct breeds for cattle, sheep, and goats in different regions (source: FAOSTAT from Scherf (2000); statistics concerning 170 countries).

		Cattle	Sheep	Goat
Africa	Population size ('000)	174 556	127 440	137 104
	Current number of breeds	251	147	89
	Number of extinct breeds	23	8	0
Asia and Pacific	Population size ('000)	461 197	408 098	390 433
	Current number of breeds	236	233	146
	Number of extinct breeds	19	7	1
Europe	Population size ('000)	162 119	185 035	26 092
	Current number of breeds	482	629	187
	Number of extinct breeds	171	142	14
Latin America and Caribbean	Population size ('000)	356 069	89 372	40 752
	Current number of breeds	107	42	34
	Number of extinct breeds	24	0	0
Near East	Population size ('000)	71 913	242 770	114 572
	Current number of breeds	86	201	94
	Number of extinct breeds	12	11	1
North America	Population size ('000)	141 481	7 891	1 428
	Current number of breeds	62	61	20
	Number of extinct breeds	5	13	1
Total population size ('000)		1 367 335	1 060 606	710 381

5.5.2 Management of Small Size Populations

Data collected within the Econogene EU project on sheep and goat diversity in marginal areas indicates the presence of significant inbreeding in most of the breeds investigated despite the scarce use of reproductive technologies (Cañon *et al.*, 2006; Peter *et al.*, 2007). This is likely due to poor breeding management of frequently small herds. An insufficient rotation of bucks/rams across farms leads to partial isolation and fragmentation at the farm, and additionally, the breed level. Hence, in addition to economic issues, and the disinterest of modern youth in agricultural careers, cultural barriers further increase the risk of loss of diversity in livestock species.

Populations with a limited number of individuals are particularly difficult to manage with the aim of maintaining an acceptable level of inbreeding. A strong social/economic network in the past allowed the exchange of parents as a source of "new blood" for restoring diversity within herds. Even during Roman times parents were actively traded and "foreign" parents were highly appreciated (Columella circa 60). Currently, several barriers to live animal trade are imposed to avoid the spread of highly infectious diseases (blue tongue, swine fever, etc.). Breeders therefore orientate their choice towards artificial insemination or parents from a few certified sources, increasing the likelihood of inbreeding. Breeders Associations could provide technical assistance to these breeders, but it is understandable that they pay more attention to high value breeds and large farms than to small size populations. The situation across Europe is however varied, with some non-profit organisations very active in sustaining small populations, such as the Rare Breeds Survival Trust in UK. The Italian Breeders Association (an organization including all high profit breeds) host herd books for smaller populations (e.g. Grigia, Burlina) and provide mating plans that avoid inbreeding. However, even such well-intentioned efforts cannot guarantee the long-term survival of all endangered breeds.

Table 5-2. Examples of effective population sizes in some cattle breeds.

Cattle breed	Country	Period	Census population size[a]	Effective population size (Ne)	Reference
Holstein	Denmark	1983-1992	-	68	Sørensen *et al.* 2005
Holstein	Germany	1999	≈ 2,200,000	52	Koenig & Simianer 2006
Holstein	Denmark	1993-2003	≈ 3,700,000	49	Sørensen *et al.* 2005
Holstein	France	1988-1991 (?)	≈ 2,500,000	46	Boichard *et al.* 1996
Holstein	USA	1999	≈ 8,500,000	39	Weigel 2001
Jersey	Denmark	1977-1991	-	87	Sørensen *et al.* 2005
Jersey	Denmark	1993-2003	≈ 640,000	53	Sørensen *et al.* 2005
Jersey	USA	1999	≈ 550,000	30	Weigel 2001
Danish red	Denmark	1977-1998	-	157	Sørensen *et al.* 2005
Danish red	Denmark	2001-2003	≈ 560,000	47	Sørensen *et al.* 2005
Japanese black	Japan	1986-1990	-	30	Nomura *et al.* 2001
Japanese black	Japan	1993-1997	≈ 530,000	17	Nomura *et al.* 2001
Montbéliarde	France	1988-1991 (?)	≈ 700,000	125	Boichard *et al.* 1996
Abondance	France	1988-1991 (?)	≈ 65,000	106	Boichard *et al.* 1996
Normande	France	1988-1991 (?)	≈ 800,000	47	Boichard *et al.* 1996
Tarentaise	France	1988-1991 (?)	≈ 14,000	27	Boichard *et al.* 1996

[a] The census population sizes were obtained either from the cited references, or from other sources such as breeder associations.

5.5.3 Threats to Adaptation

Adaptive traits may be rapidly lost by poorly designed crossbreeding leading to dilution of local genetics by exotic germplasm. Crossbreeding to a more productive breed from elsewhere, most often a high production breed, is widespread and can destroy the specific features of an indigenous breed within a few generations. The case of trypanotolerant livestock breeds in West Africa represents a good example of local adaptation that might be disrupted by crossbreeding (Agyemang, 2005). Recovery from such loss of distinctiveness can be extremely difficult, requiring many generations of backcrossing to purebred indigenous animals. In some cases recovery is impossible because no purebred animals remain to allow a backcrossing recovery program (for instance, there are so few pure breed Maremmana cattle remaining today in Central Italy, that even crosses are granted the label of certification of origin). A number of examples exist, particularly in developing countries, where indiscriminate repeated cross-breeding quickly disrupted generations of natural and anthropic selection for adaptation to harsh environments.

Traits such as resistance to local infectious and parasitic diseases, adaptation to poor forage, homing and gregarious behaviour, which represent key traits for the survival and management of herds in extensive farming, can be rapidly lost and difficult to rescue. An example of this effect can be found in Corsica, where local goats, when crossed to Alpine or Saanen breeds loose their gregarious and homing behaviour and get lost in the mountains where they are raised in free range. Another example is the Red Maasai sheep in Kenya, renowned for its hardiness and disease resistance, especially its resistance to gastrointestinal parasites (Baker *et al.*, 1998). In the mid 1970's a subsidised dissemination program for Dorper rams was established in Kenya. Widespread indiscriminate crossbreeding followed. No instructions were supplied to farmers about how to maintain a continuous crossbreeding program and many farmers continued crossing their flocks to Dorpers, which subsequently proved unsuitable in many production areas (Council, 2005).

5.5.4 Geographic Confinement

When the traditional rearing area is geographically limited, an additional risk is represented by highly contagious infectious diseases that may wipe out an entire population if back-ups do not exist elsewhere. This was the case of the Herdwick sheep breed in UK, almost exterminated recently by the foot and mouth disease epidemics in 2001. (Alderson, 2001)

Several methods are proposed for conservation of farm animal genetic resources. They may be *in vitro,* through the cryo-preservation of animal gametes, embryos and tissues or *in vivo*, by conserving animal flocks *ex-situ,* that is outside their place of origin, for example in experimental farms, or *in situ*, that is within their natural environmental and socio-economic context. When the conservation of adaptive traits in a changing environment is the actual aim, *in situ* conservation is the best option.

5.6 Conclusion

Domestic animals are currently losing genetic diversity through many mechanisms. First, the highly productive breeds have recently been intensively selected for production traits, without enough emphasis on the preservation of the overall genetic diversity. Many breeds in developed countries suffer from a very low effective population size despite their total number of individuals. The strong decrease in fertility of the Holstein cattle, as well as the recent emergence of new hereditary diseases, is a sign that inbreeding is becoming a serious threat in the short term. Second, autochthonous breeds in marginal areas are also seriously endangered. Farmers are often obliged to abandon their traditional breeds and to raise more competitive industrial breeds. As a consequence, many locally adapted breeds have already disappeared (Table 5-1). Furthermore, even in less developed countries, the introgression of genes from industrial breeds seriously compromises the long-term persistence of genetic resources in locally well-adapted breeds.

Many parallels can be found in issues related to threats and conservation of domestic and wild animal species. One of the most problematic threats to wild populations is the fragmentation due to human activity (Frankham *et al.*, 2002). Habitat fragmentation induces the risk of excessive genetic drift and inbreeding in isolated populations. In domestic species, fragmentation is mainly due to human intervention that blocks gene flow

across populations by keeping breeds as separate breeding units. In non-industrial breeds the diffused cultural inability to properly manage small size populations may lead to fragmentation and isolation even at the farm level.

In conservation biology, it is well known that the long-term viability of populations is directly linked to its effective population size. A reduction of the effective population size to below 50 seriously compromises the short-term survival of a wild population. This problem is exacerbated in industrial domestic breeds.

The real value of biodiversity is difficult to assess. This is true for wild species (e.g. (Myers, 1996)), but also for domestic animals. Most of the difficulties in preserving the diversity of domestic animals are due to a short-term evaluation of the economic value that promotes the exclusive use of industrial breeds. Furthermore, preservation of genetic resources in domestic animals does not have the same image for the public as preserving the giant panda or whales. Domestic animals have been selected and modified by humans. They do not bear the same "natural" perception that wild species have for the public, despite being our food. This is a paradox, because our future will undoubtedly be linked to our ability to produce food from domestic animals. The fact that domestic animals are numerous, and that we have full control on their reproduction make it difficult to explain that some breeds are endangered and that we are losing valuable genetic resources.

In light of the current loss of genetic diversity in farm animals, it is extremely important to take measures that promote a sustainable management of these genetic resources. These measures must prioritize the *in situ* preservation of endangered breeds, and selection programs that will restore the genetic diversity in industrial breeds. *Ex situ* conservation is not suitable, as it relaxes the traditional selection pressures and would not allow the preservation of the genetic resources of interest. In the same way, cryo-conservation should only represent a very short-term strategy in case of emergency. The situation is exacerbated by the fact that we do not know which feature will be useful to exploit in the future, and which breed carries this feature today.

Beside the sustainable management of domestic species themselves, it is also extremely important to take care of the wild relatives and of the wild ancestors when they still exist. The wild ancestor of cattle is already extinct, and the closest wild relatives are vulnerable (*Bos frontalis*), endangered (*B. javanicus*), or critically endangered (*B. sauveli*); the putative wild ancestors of sheep and goats are all vulnerable or endangered (according to IUCN classification).

Concerning less productive breeds, the price of their products should take into account their value as storage of unique genetic diversity. The public should be made aware of this before any strategies for the sustainable management of livestock genetic resources are implemented. Therefore, in opposition to the rules of the global market, subsidies should be given to help farmers who contribute to the preservation of genetic resources in marginal or rare breeds. The Doha agreement (World Trade Organization, 2001) took this issue partially into consideration in permitting state subsidies for typical agricultural products. However this decision was only taken because of the marginal volume of this niche in comparison to the overall market.

Although cattle, sheep, and goats cannot be considered as endangered species according to the number of individuals, it is clear that many breeds are highly endangered, and that we are losing genetic resources. In a few decades, we might lose most of the highly valuable genetic resources that humanity has gradually selected over the past 10,000 years. Despite many conservation programs implemented by the FAO, the conservation of many locally adapted breeds opposes the short-term economic profit. Sadly, the erosion of livestock genetic resources is still continuing, and the same observation has also been made for crops (Esquinas-Alcazar, 2005).

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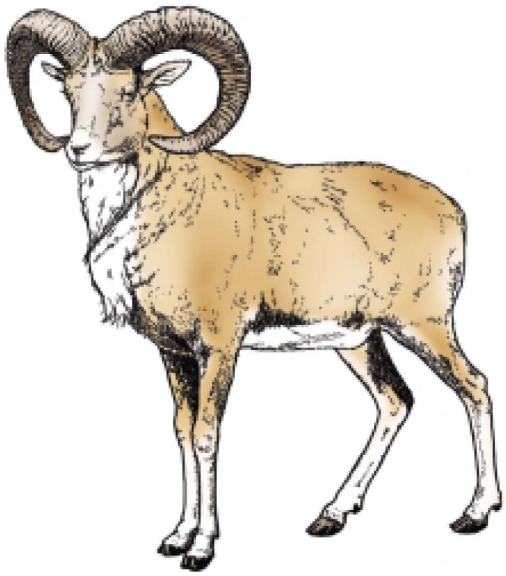

Conclusion and Perspective

The evolution of the *Ovis* genus has been poorly understood until now, and different classifications including from one to seven species have been proposed in the last two decades. Some classifications are based on the classical morphological concept of species, while other use biological approaches that stress chromosomal and molecular uniqueness. The most complex situation occurs in Central Iran where there is a hybridization zone between the Mouflon and the Urial sheep. Even though they have, different chromosome numbers which; they produce fertile offspring.

The topology of the tree generated from mtDNA sequence data shows that wild sheep have evolved into two major monophyletic groups. The first clade is the Pachyceriform group that has been previously defined on morphological criteria and that enclose *O. nivicola*, *O. canadensis* and *O. dalli*. The second clade, which we define here as the Asiaticform group, is composed of two groups that have been previously defined: the monophyletic Argaliform group (*O. ammon*), and the Moufloniform group (*O. orientalis* and *O. vignei*) that appears to be paraphyletic. Moreover, our data show that the European mouflon belongs to the *O. orientalis* clade, and thus may be considered as a subspecies of *O. orientalis* (i.e., *O. orientalis musimon*) and not as a species by itself. However, there is no fossil record of wild sheep before 5000 years ago in Europe, suggesting the mouflon came with human at the Neolithic period. This is confirmed by the genetic proximity between the European mouflon and the domestic sheep.

Human has domesticated very few livestock species. The most common ones are cattle, sheep, goats, pigs, horses and buffal*o*. The questions of how, when, where and why people first domesticated the animals are central for understanding the history of humanity. The origin of domestic sheep is controversial with three putative ancestral species and two potential domestication areas. We based our study on the origin of domestic sheep on an extensive sampling of both the sheep and the wild species. This allowed to compare the genetic diversity of domestics and wilds by using the mtDNA phylogeny, and to confront the results obtained to archaeological data. Our results showed the Asiatic mouflon (*O. orientalis*) is the only true ancestor of domestic sheep. In addition, based on the geographic distribution of the haplotypes of *O. orientalis* that are close to the domestic haplotypes, we demonstrates that the sheep has only been domesticated in Eastern Anatolian and Northern Zagros mountains, with no contribution of the Indus Valley. The domestication process would have started by the protection of wild sheep populations leading to reduce the impact of predators. Dog, as the first domesticated animal, could have helped men for this protection.

Our study does not support the fact that the several domestic haplogroups have been domesticated from different wild subspecies (Hiendleder, 2002). *O. orientalis gmelini* was the only subspecies involved in a successful domestication process. In addition, our results show that the domestication of two haplogroups (A and B) happened in the same region of Eastern Anatolia and that the other haplogroups (C, E and maybe D) have been domesticated in Northern Zagros. These two phenomena occurred independently, and the domestication has begun earlier in Anatolia than in Zagros. Now, there are only sheep from the A and B haplogroups in Europe while several other haplogroups are represented in the Middle East, Asia and in Northern Africa. This present geographic distribution of domestic haplogroups strongly suggests that the first domesticated sheep were brought to Europe from Eastern Anatolia along the North of the Mediterranean Sea. The transfer of sheep by humans would also have contributed to bring new haplotypes in wild populations, when domestic sheep became feralized. Our study suggest that some haplotypes found in *O. orientalis anatolica* in Western Anatolia could come from the populations of Eastern Anatolia and Northern Zagros Mountain.

Ecosystems and species biodiversity are decreasing due to human activities. The risk of species extinction could be reduced by genetic management regimes. In this context, the preservation of wild species that are close to domestic species is important because they constitute a genetic resource. Actually, the livestock genetic resources are including all domestic breeds and their wild relatives. Animal genetic resources are important to the survival of a large number of people in the pastoral world, but also for agro-food industries. In developing countries, they represent an important source of high quality protein and overall economic development. The extinction of a breed or a population means the loss of unique adaptive attributes, which are often under the control of many interacting genes and are the result of complex interactions between the genotype and the environment. Thus, the conservation and protection of genetic resources is a real concern, all the more because the genetic diversity of domestic breeds is often reduced. Domestic breeds have undergone strong selection pressures by human, and many breed in developing countries have been founded by a low number of individuals and are highly inbred. This could lead to a decreasing fertility or to a lower resistance to several diseases. Furthermore, the decline of traditional livestock production systems and the replacement of local genetic resources by exotic high-performing breeds are another source of problems in developing countries.

We need to develop strategies for the sustainable management of these resources. These should include conservation genetic approaches as well as the development of new ways of using resources. Molecular characterization can play a role in uncovering the history, and estimating the diversity and genetic structure of animal genetic resources. It can also help for the genetic management of small populations, in order to avoid strong inbreeding. Although cattle, sheep, and goats cannot be considered as endangered species according to the number of individuals, it is clear that many breeds are highly endangered, and that we are losing genetic resources. In a few decades, we might lose most of the highly valuable genetic resources that humanity has gradually selected over the past 10,000 years.

Future Research directions

Our results provide many indications on where future research should focus. It is clear that there is still much to be done in order to understand the genetic and phylogeny of the *Ovis* genus. In order to complete the taxonomic results obtained with mtDNA data, the study of nuclear gene is required. While mtDNA provides a "maternal" view of the evolutionary history of the *Ovis* genus, the study of paternally inherited genes could bring new information. The use of new molecular markers would also help testing the validity of several subspecies previously described on the base of their morphological characters and geographic distribution. Moreover, the use of microsatellite markers or AFLP would be useful for estimating gene flows between wild populations and understanding their genetic structure. The results of such studies could be used in conservation genetic programs for example in allowing the identification of threatened populations. Because these wild populations constitute a genetic resource for domestic sheep, it will be necessary to develop protection areas and a conservation programs.

When considering the study of the sheep domestication history, we need more studies for finding the archaeological sites in the present and ancient distribution of the Asiatic mouflon (*O. orientalis*). In addition, this could help collecting ancient samples for comparing with present data. For instance, a genome scan on nuclear gene would allow detecting the mutations differentiating wild sheep from ancient and present domestic sheep. Then, the genes that have been selected during the domestication process could be identified.

The quality and impact of these future studies will strongly lie on the quality of the sampling. Thus, it will be necessary to extend the sampling of domestic and wild sheep to areas that have been poorly explored until now. The main areas that remains to be studied are the Africa for domestic sheep and several parts of Asia for wild species.

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Abstract

The systematic of the *Ovis* genus has been difficult to establish with several classifications proposed. Seven main groups of wild sheep are distinguished on the basis of different karyotype, morphologies and geographic distributions. The present work provides new insights for the systematic and evolution of the wild sheep by performing cytochrome *b* phylogenies inferred from Bayesian, maximum likelihood, and neighbour joining methods. These phylogenies were based on 267 samples covering the whole geographic distribution area and representative of most of the wild sheep subspecies. In this phylogeny urial and mouflon, which are either considered as a single species (*Ovis orientalis*) or as two separate species (*O. orientalis* and *O. vignei*), form two monophyletic groups strongly supported by high bootstrap values. Hybrids between *O. vignei* and *O. orientalis* appear in one or the other group, independently from their geographic origin within the hybrid zone. The European mouflon *Ovis musimon* is clearly in the *O. orientalis* clade. The other species, *O. dalli*, *O. Canadensis*, *O. nivicola* and *O. ammon* are monophyletic. Three of these wild species (*O. orientalis, O. vignei and O. ammon*) has been considered as potential ancestors of the domestic sheep until now. The phylogenetic relationships between the domestic sheep these three Asiatic wild species demonstrate that the Asiatic mouflon (*O. orientalis*) is the only true ancestor. The comparison of the mitochondrial DNA diversity of 130 domestic sheep with that of 140 Asiatic mouflons from all over its modern distribution area allows restricting the cradle of domestication between Eastern Anatolia and the Zagros mountains, clearly excluding the Lower Indus Valley and more Eastern Asiatic regions. A large part of the wild genetic diversity has been captured, which indicates a large effective population size at the time of domestication. This challenges the current believe suggesting the occurrence of bottlenecks at the beginning of the domestication process.

Résumé

La systématique du genre *Ovis* est restée confuse jusqu'à aujourd'hui, et plusieurs classifications ont été proposées. Sept principaux groupes de moutons sauvages sont distingués sur la base de différences caryotypiques, morphologiques et géographiques. Le présent travail fournit de nouvelles données sur la systématique et l'évolution du genre *Ovis*, à partir de phylogénies de Cytochrome b. Ces phylogénies sont basées sur l'analyse de 267 échantillons représentatifs de la plupart des sous-espèces d'*Ovis* et de l'ensemble de leur aire de répartition. L'Urial et le Mouflon, qui sont considérés soit comme une seule espèce (*Ovis orientalis*) soit comme deux espèces séparées (*O. orientalis* and *O. vignei*), forment un groupe monophylétique fortement soutenu. Les hybrides entre *O. vignei* et *O. orientalis* apparaissent dans l'un ou l'autre des groupes, indépendamment de leur origine géographique au sein de la zone hybride. Le mouflon européen (*O. musimon*) appartient clairement au clade d'*O. orientalis*. Les autres espèces, *O. dalli*, *O. Canadensis*, *O. nivicola* et *O. ammon* sont monophylétiques. Trois des espèces sauvages, *O. orientalis, O. vignei* et *O. ammon*, ont été considérées comme pouvant être à l'origine du mouton domestique. Les relations phylogénétiques entre ces espèces et le mouton montrent maintenant que le seul véritable ancêtre est le mouflon asiatique (*O. orientalis*). La comparaison de la diversité mitochondriale de 130 moutons avec celle de 140 mouflons asiatiques représentatifs de l'ensemble de l'aire de répartition, permet de localiser l'aire de domestication. Elle s'étend de l'est de l'Anatolie aux monts Zagros, et exclue la basse vallée de l'Indus et l'Asie de l'Est. Une grande partie de la diversité génétique sauvage a été capturée, indiquant des tailles efficaces de population importantes au moment de la domestication. Ceci remet en question l'existence couramment admise de goulots d'étranglement au début de la domestication.