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Reliability of mouflon aging using morphological characteristics from observations in the field

Mathieu Garel, Jean-Marc Cugnasse & Daniel Maillard

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In mouflon *Ovis gmelini musimon* × *Ovis* sp. populations, different morphological characteristics are often used to discriminate among age classes from observations in the field, such as horn size and horn development for males and size of white facial mask for females. In the Caroux-Espinouse mouflon population, we used linear discriminant analysis to test the ability of these two commonly used morphological characteristics to discriminate among age classes in the field. Models were developed using measurements taken from 643 mouflons harvested between 1977 and 1985. Classification ability of the horn length for males was poor (77.5%, SD = 15.0, N = 404) but better than facial mask length for females (40.1%, SD = 27.8, N = 212). Male lambs were the only age class successfully classified (≥ 90%). As reported elsewhere, we found that facial mask length was not a reliable predictor of male age (classification ability: 40.3%, SD = 21.7, N = 431). In the Caroux-Espinouse population, horn length and facial mask length were poor indicators of age. Our results suggest an important need to evaluate the reliability of criteria used in mouflon populations to discriminate among age classes from observations in the field. We recommend working with a restricted number of age classes and studying a combination of behavioural and physical characteristics.

Key words: age discrimination, Caroux-Espinouse massif, discriminant model, facial mask, horn length, mouflon, Ovis gmelini musimon × Ovis sp.

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Age assessment is an important prerequisite for most population studies because age is known to influence most life history traits such as reproductive success and survival of vertebrates (Stearns 1992). In ungulate populations, which are strongly age-structured (Gaillard et al. 2000, Coulson et al. 2001), knowledge of age distribution is important to evaluate management programmes (Bender et al. 1994, Jensen 1996, Van Deelen et al. 2000, Sæther et al. 2001). Therefore errors in age estimation can lead to erroneous conclusions when assessing the

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demographic status of a population and deriving harvest strategies.

Most reliable methods of age determination were developed to estimate the age of animals that had been captured (Morris 1972). However, studied ecological and ethological processes or derived harvest programmes often require the ability to distinguish age classes from observations in the field (e.g. Gray & Simpson 1980).

In the mouflon *Ovis gmelini musimon* × *Ovis* sp., age classes were principally discriminated using length and horn development for males or size of white facial mask for females (Türcke & Schmincke 1965, Rieck 1975, Tomiczek 1989, Ludwig & Peukert 1992). Although numerous field studies have used these criteria to estimate age when analysing age-related behavioural and ecological characteristics of mouflons (e.g. Pfeffer 1967, Tomiczek 1989, Bon et al. 1993, Le Pendu et al. 1995, Ciucci et al. 1998, Réale & Boussès 1999), their validity remains largely untested. Only Boussès & Réale (1994) have proved that facial mask length did not accurately assess female age in the Kerguelen mouflon population. However, considering the diversity of genetic origin of mouflon populations (for a review see Cugnasse 1994), it is difficult to extrapolate their results to indigenous mouflon populations (e.g. Corsican). Moreover, although several authors (e.g. Pfeffer 1967, Tomiczek 1989) have suggested that facial mask length is not a reliable predictor of male age because it displays great inter-individual variability, no one has tested the validity of this suggestion.

We therefore tested the reliability of horn length (males) and facial mask length (males and females) when used to discriminate age classes in the field in the Caroux-Espinouse mouflon population. We developed a linear discriminant model using measurements from 643 mouflons (431 males and 212 females) harvested during 1977-1985. Using simulations, we assessed the proportion of well-classified animals within each age class obtained from horn and facial mask lengths. We then discuss the reliability of these criteria and make recommendations for future studies of unmarked free-ranging mouflons.

Material and methods

Study area

The mouflon population inhabiting the Caroux-Espinouse massif (43°40'N, 3°0'E) has been monitored by the Office National de la Chasse et de la Faune Sauvage since 1974. The Caroux-Espinouse massif is situated on the soutern border of the Massif Central in southern France. Climatic conditions are variable, consisting of a mixture of oceanic, Mediterranean and mountain influences (Thiebaut 1971). The vegetation cover is a mosaic of beech *Fagus sylvatica*, chestnut *Castanea sativa* and evergreen oak *Quercus ilex* forests moving from north to south, heather *Calluna vulgaris*, *Erica cinerea* and broom *Cytisus purgans*, *Cystus scorparius* moorlands, and rocky slopes.

The population has grown from 19 individuals introduced during 1956-1960 to the national wildlife reserve (Cugnasse & Houssin 1993), situated in the central part of the Massif. Mouflons originated from both native Corsican *Ovis gmelini musimon* and continental mouflon (French national reserve of Chambord and Czechoslovakia). Therefore, our study population is of uncertain and possibly mixed origin, so we use the denomination of *Ovis gmelini musimon* × *Ovis* sp. following the recommendations of Cugnasse (1994). Hunting (by stalking and beating) occurred from September to February.

Measuring morphological characteristics of harvested animals

Horn length and facial mask length were taken from a sample of 643 mouflons harvested during 1977-1985. Ewes collected were divided into five age classes based on the number of permanent lower incisors present (Rieck 1975). Tooth eruption and replacement were the only reliable criteria used to estimate the age of females. When performed during the hunting season (October-February) it allowed a reliable estimation of age until 3½ years (Rieck 1975). During this period, five stages of tooth eruption may be described: no permanent incisor for lambs, two permanent incisors for yearlings, four permanent incisors for 2½ year olds, six permanent incisors for 2½-3½ year olds, and eight permanent incisors for ≥ 3½ year olds (Rieck 1975). Age determination of males was performed using the horn annulus technique (Geist 1966) which is based on counts of horn growth annuli. Horn growth annuli correspond to drastic reduction of linear horn growth which results from seasonal difference in the nutritional plane due to variations in forage quality (Türcke & Schmincke 1965, Pfeffer 1967, Rieck 1975, Gray & Simpson 1985) or from hormone-induced factors related to the reproductive cycle (Hoefs 1982, Lincoln 1998).

Horn size and horn development were principally used for male aging from observations in the field. Age classes were characterised by the position of the horn tip related to the base of the neck and the eyes (Türcke & Schmincke 1965, Rieck 1975, Chauvière 1978, Tomiczek 1989). We measured horn length on 404 harvested males from base to tips of horns following the external side with a flexible steel tape. We kept the measure of the longest

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or least damaged horn rather than the mean length of horns, because some horns were broken (3%).

The size of the white facial mask is the most often reported criterion for age determination of ewes from observations in the field (Türcke & Schmincke 1965, Pfeffer 1967, Tomiczek 1989, Boussès & Réale 1994). The facial mask length corresponds to a whitening of the skin of the face, which progresses with age from the nostril towards the face (Tomiczek 1989). The facial mask length was measured on 212 harvested females along the median axis of the face from the upper side of the nostril. Facial mask length was also measured for males (N = 431) to test if, as suggested elsewhere (e.g. Pfeffer 1967, Tomiczek 1989), this criterion is not a reliable predictor of male age. Horn length was not a reliable criterion for age determination of females which are mostly hornless (79.1%; M. Garel, unpubl. data).

Relation between exact age and morphological characteristics

We used data collected between 1 October and 1 March when linear horn growth is reduced (Pfeffer 1967, Hoefs 1982, Lincoln 1998), and facial mask size is the most obvious (Pfeffer 1967).

We used a linear model to study the relationship between exact age of males and horn size. We then built a linear discriminant model (Krzanowski & Marriot 1994) to evaluate the ability of horn length and facial mask length to discriminate between age classes of animals. In Caroux-Espinouse, the following age classes were recognised from observations in the field for both sexes: lambs, yearlings (1 year old), 2-3 year olds, 4-6 year olds, and +6 year olds (based on Pfeffer 1967 and Chauvière 1978). For females, it was not possible to estimate the exact age of the animal beyond three years of age, so we worked directly with the five tooth classes described above (for a similar approach see Boussès & Réale 1994).

The linear discriminant procedure (R/MASS's lda procedure; R Development Core Team 2004) computes linear functions for classifying observations into the five age classes (males) or the five tooth classes (females) on the basis of horn length or facial mask length (Venables & Ripley 2002). The linear discriminant model was built using 60% of the data set. The ability of morphological characteristics (horn length and facial mask length) to accurately discriminate between mouflon age classes was assessed by calculating the proportion of well-classified individuals using the remaining data (40%). We performed bootstrap simulations to estimate an average success rate (with standard deviation) based on 1,000 linear discriminant functions built from 1,000 random samples using 60% of the data set. We reported the mean number (with standard deviation) of animals used to estimate within age-class success rate. A correct overall and within age class success rate of ≥ 90% was considered adequate for model performance (for a similar approach see Bender et al. 1994). The likelihood of successful classification using discriminant models can be influenced by the a priori probabilities of an observation belonging to any of the discriminant classes (i.e. the prior probabilities of membership in each class). To address this problem, we assumed equal probability of mouflon belonging to any age class (probability = 0.2 for all five age classes and stages of tooth eruption).

All analyses and resampling procedures were performed using R 1.8.0 (R Development Core Team 2004).

Results

Reliability of horn length when used to estimate age of males

Horn length increased rapidly until the animals were six or seven years of age (Fig. 1), and then increased very slowly (intercept = 258.75 (SE = 9.58), $slope_{age}$ = 118.65 (4.24), $slope_{age^2}$ = -6.65 (0.41)). Differences among age

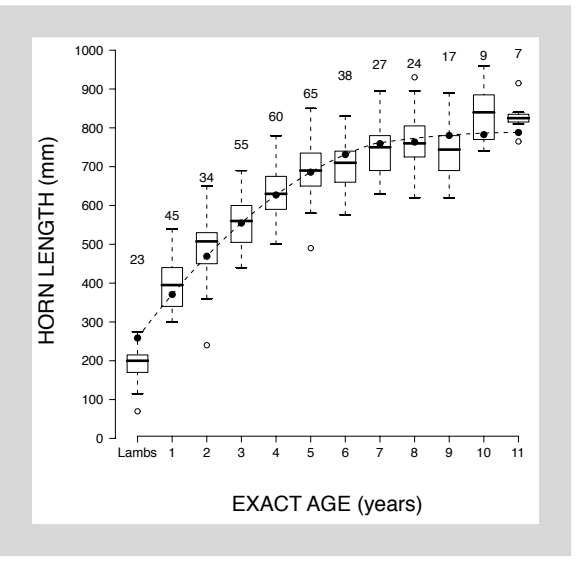


Figure 1. Relationship between horn length (in mm) and age (in years) of males in the Caroux-Espinouse mouflon population. Length of the longest horn was measured on 404 males harvested during 1977-1985. The boxes indicate, from bottom to top, the first, the median and the third quartile; the vertical lines indicate the most extreme data points which are no more than 1.5 times the interquartile range from the box; the open circles correspond to data out of this range (Emerson & Strenio 1983). Figures above the plots give the sample sizes of each age class. The fitted quadratic model (dashed line) is shown (for curve's parameters, see the text).

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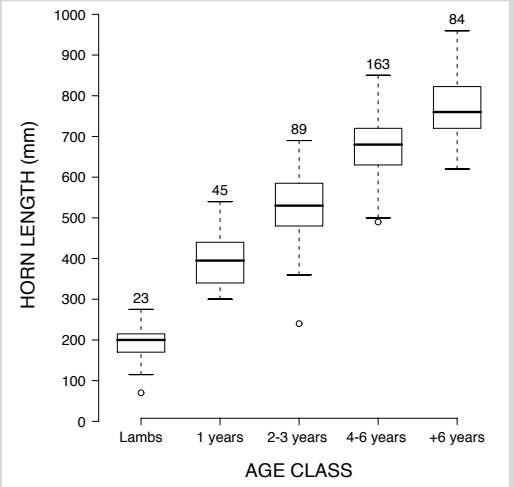


Figure 2. Relationship between horn length (in mm) and age classes of males determined from field observations in the Caroux-Espinouse mouflon population. Length of the longest horn was measured on 404 males harvested during 1977-1985. The boxes indicate, from bottom to top, the first, the median, and the third quartile; the vertical lines indicate the most extreme data points which are no more than 1.5 times the interquartile range form the box; the open circles correspond to data out of this range (Emerson & Strenio 1983). Figures above the plots give the sample size of each age class.

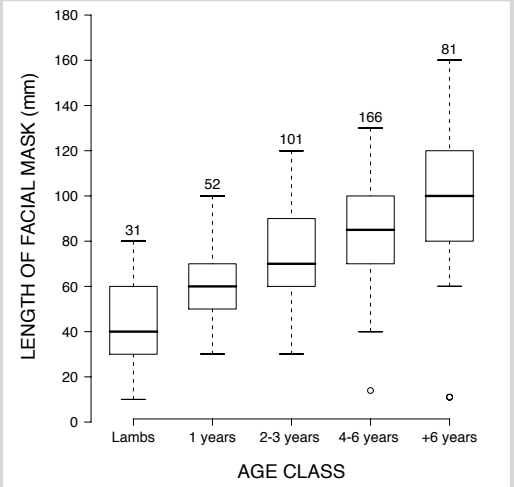


Figure 3. Relationship between facial mask length (in mm) and age classes of males determined from field observations in the Caroux-Espinouse mouflon population. Facial mask length was measured on 431 males harvested during 1977-1985. The boxes indicate, from bottom to top, the first, the median, and the third quartile; the vertical lines indicate the most extreme data points which are no more than 1.5 times the interquartile range form the box; the open circles correspond to data out of this range (Emerson & Strenio 1983). Figures above the plots give the sample size of each age class.

classes accounted for 80.4% of the variability of horn length (Fig. 2). However, animals older than six years may have a horn length similar to that of an animal 2-3 years of age. Inter-individual variability was lower for lambs than for the other age classes. The performance of the linear discriminant function was poor (Table 1). Overall, success rate was 77.5% (15.0). Success rates

Table 1. Success rate of age class determination based on the horn length discriminant function developed for 404 males harvested in Caroux-Espinouse from 1977 to 1985. The average success (± SD) within age class and the average number (± SD) of animals used to estimate those rates were calculated based on 1,000 linear discriminant functions built from 1,000 random samples using 60% of the data set. The remaining data (40%) were used to estimate the success classification rate within age class and the related sample size. *A priori* probabilities of an observation belonging to any of the discriminant classes was 0.2.

Age class[a]	Success rate		Sample size	
Lambs	100.0	(0.2)	9.2	(2.3)
1 year	84.5	(8.3)	18.0	(3.2)
2-3 years	68.5	(6.9)	35.7	(4.2)
4-6 years	60.1	(5.2)	65.4	(5.1)
+6 years	74.4	(7.1)	33.7	(4.1)
Total	77.5	(15.0)	32.4	(19.5)

[a] age determination was performed using the horn annulus technique (Geist 1966).

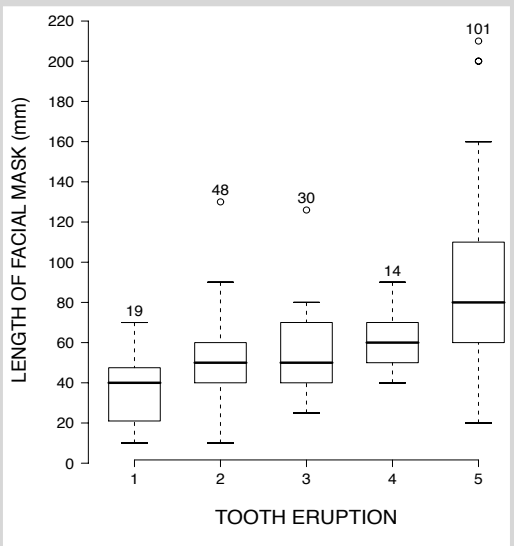


Figure 4. Relationship between facial mask length (in mm) of females and tooth eruption class (1 = no permanent incisor, 2 = two permanent incisors, 3 = four permanent incisors, 4 = six permanent incisors and 5 = eight permanent incisors; see Rieck 1975) in the Caroux-Espinouse mouflon population. Facial mask length was measured on 212 females harvested during 1977-1985. The boxes indicate, from bottom to top, the first, the median, and the third quartile; the vertical lines indicate the most extreme data points which are no more than 1.5 times the interquartile range form the box; the open circles correspond to data out of this range (Emerson & Strenio 1983). Figures above the plots give the sample size of each age class.

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Table 2. Success rate of age class determination based on the facial mask length discriminant function developed for 431 males harvested in Caroux-Espinouse from 1977 to 1985. The average success (± SD) within age class and the average number (± SD) of animals used to estimate those rates were calculated based on 1,000 linear discriminant functions built from 1,000 random samples using 60% of the data set. The remaining data (40%) were used to estimate the success classification rate within age class and the related sample size. *A priori* probabilities of an observation belonging to any of the discriminant classes was 0.2.

Age class[a]	Success rate		Sample size	
Lambs	66.0	(13.5)	12.4	(2.6)
1 year	23.6	(10.1)	21.0	(3.3)
2-3 years	19.5	(8.6)	40.5	(4.3)
4-6 years	31.6	(8.9)	66.7	(5.0)
+6 years	60.9	(8.9)	32.3	(3.9)
Total	40.3	(21.7)	34.6	(19.2)

[a] age determination was performed using the horn annulus technique (Geist 1966).

ranged from 100% (0.2) for lambs to 60.1% (5.2) for 4-6 year olds. According to our criteria (≥ 90%) lambs were the only age class well classified (see Table 1).

Reliability of facial mask length when used to estimate age of males and females

Differences among age classes accounted for only 33% of variability in facial mask length of males (Fig. 3) and females (Fig. 4). For example, lamb facial mask length (age class 1) was often similar to that of ewes ≥ 3½ years of age (age class 5; see Fig. 4). The performance of the linear discriminant functions was similar for males and females (Tables 2 and 3) but was lower than that obtained for horn length discriminant function (see Table 1).

Table 3. Success rate of age class determination based on the facial mask length discriminant function developed for 212 females harvested in Caroux-Espinouse from 1977 to 1985. The average success (± SD) within age class and the average number (± SD) of animals used to estimate the rates were calculated based on 1,000 linear discriminant functions built from 1,000 random samples using 60% of the data set. The remaining data (40%) were used to estimate the success classification rate within age-class and the related sample size. *A priori* probabilities of an observation belonging to any of the discriminant classes was 0.2.

Age class[a]	Success rate		Sample size	
1	67.0	(17.0)	7.5	(2.1)
2	22.5	(14.3)	19.2	(3.1)
3	8.7	(11.8)	12.1	(2.5)
4	39.3	(25.1)	5.5	(1.8)
5	63.5	(6.8)	40.7	(3.7)
Total	40.1	(27.8)	17.0	(12.9)

[a] age classes were based on tooth eruption and replacement according to Rieck (1975). The following age classes were used: 1) no permanent incisor, 2) two permanent incisors, 3) four permanent incisors, 4) six permanent incisors, and 5) eight permanent incisors.

Overall, success rate was only 40.3% (21.7) and 40.1% (27.8) for males and females, respectively. According to our criteria none of the age classes was successfully identified (see Tables 2 and 3).

Discussion

Our results are consistent with a former study (Boussès & Réale 1994) on the Kerguelen mouflon population which found that facial mask length cannot be used to discriminate among female age classes, because substantial individual heterogeneity occurs. Moreover, as suggested elsewhere (e.g. Pfeffer 1967, Tomiczek 1989), we found that facial mask length is not a reliable predictor of male age because it displays great variability between animals. Inter-individual variation in horn length may also be problematic when assessing age class, considering that field workers estimate age classes with the position of the horn tip related to the base of the neck and the eyes. Thus, we think that inter-individual variability in horn and facial mask lengths may account for the poor performance of these criteria to determine age class in both sexes.

Inter-individual variation may result from the especially large intra-specific genetic variability of this population (Petit et al. 1997) which has ancestors crossbred with other wild or domestic sheep (for a review see Cugnasse 1994). Indeed crossbreeding may increase phenotypic variability (Cugnasse et al. 1998). The confounding effect of differences in annual resource availability or of changing range condition (Gray & Simpson 1985) may also account for the variability observed in facial mask and horn length for a given age. Horn development may be more affected by forage quality and environmental conditions (Bunnell 1978) than genetics (Hook 1998). Therefore, temporal (e.g. closure of habitat, density variations) and spatial variations (e.g. latitude, altitude) in habitat conditions may involve great inter-individual and inter-population variability in horn length, limiting considerably the use of this age criterion. For example, in a Urial *Ovis orientalis arkal* population intensive live stock grazing seems to have reduced available food, and thus horn growth rate, involving possible mistakes in age class estimation from observation in the field (Frisina 2002).

Environmental and genetic variations within and between mouflon populations (Cugnasse 1994, Weller 2001), call for caution when assigning male age classes using horn length. Such variations involve great differences in horn growth patterns between European populations (Hoefs 1982). Rams from Mediterranean popu-

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lations experience more horn growth during their first year of life than rams from northern populations (Hoefs 1982, Hoefs & Hadjisterkotis 1998). In our study area, rams had already reached 70% of total horn length at the age of three (75% in the Corsican mouflon (Pfeffer 1967) and in the Cyprus mouflon *Ovis gmelini ophion* (Hoefs & Hadjisterkotis 1998)). Horn growth is thus very slow beyond three years of age. Moreover, by eight years of age the wear of the horn tip of mouflon rams often exceeds new growth at the horn base (Hoefs 1982). The quadratic relationship (see Fig. 1) between age and horn length suggests that the usefulness of horn length in estimating mouflon age from observations in the field may be limited in practice and contribute to explain the relatively poor performance of horn growth to distinguish among animals ≥ 2 years old reported here.

Observed effect related to age estimation may contribute to the inter-individual variability. A reliable estimation of age for a given male assumes that one growth annulus is produced each winter (Geist 1966, Hoefs & Robert 1984, Gray & Simpson 1985). Mediterranean conditions may, however, reduce the detectability of horn growth annuli and therefore increase among-observer differences in age estimates (Hoefs & Robert 1984, Gray & Simpson 1985). For females, it was not possible to estimate the exact age of an animal, and therefore animals of different ages were pooled for the fourth (2½ and 3½ year olds) and the fifth classes (≥ 3½ year olds) determined by tooth eruption. This methodological bias related to age estimation of females also contributes to the inter-individual variability recorded.

Facial mask length and horn size have often been reported to be reliable aging criteria for age assessment in mouflons of both sexes (see Tomiczek 1989). However, our results did not support this assertion. In particular, facial mask length was a poor predictor of age in females of the Caroux-Espinouse population. Therefore, there is an important need to reconsider criteria used to assess age classes from observations in the field when studying mouflon populations, especially for females. We recommend using a restricted number of age classes: < 1 year old (noted lambs) and other (noted ewes and rams according to sex). To distinguish lambs from other mouflons, we recommend principally to use a combination of behavioural characteristics, such as mother-lamb relationship (sucking during the first months), and physical characteristics, such as age-related variation in body size and morphological configuration (e.g. rib-cage depth and head shape; see Chauvière 1978 and Tomiczek 1989). We recommend that growth pattern descriptions rather than colouration patterns (facial mask) be used to assess proposed age classes.

Considering the importance of age assessment when studying ungulate population dynamics (Gaillard et al. 2000), and thereby the setting of management programmes (Jensen 1996, Sæther et al. 2001), we recommend, whenever possible, that free-ranging ungulates be marked during their first year of life when age determination is accurate (e.g. Gaillard et al. 1997 on roe deer *Capreolus capreolus*).

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