Centric Fusion Polymorphisms in Waterbuck (Kobus ellipsiprymnus)


Twenty-six captive individuals of the *ellipsiprymnus* subspecies group of *Kobus ellipsiprymnus* were found to have chromosomal complements of \(2n = 50-52\) (FN = 61–62), and 26 of the *defassa* subspecies group, including three specimens from Lake Nakuru National Park, Kenya, had complements of \(2n = 53-54\) (FN = 62). G-banded karyotypes that were numbered according to the standard karyotype of *Bos taurus* revealed that variation in diploid number was the result of polymorphism for two independent centric (Robersonian) fusions. The *ellipsiprymnus* group was polymorphic for a 7;11 centric fusion. Both elements of chromosome pairs 7 and 11 were fused in fusion homozygotes (\(2n = 50\)); in fusion heterozygotes (\(2n = 51\)), only one element of each pair was fused. The 7;11 fusion was lacking in specimens with \(2n = 52\). The *defassa* group was polymorphic for a 6;18 centric fusion; individuals were either heterozygous for the fusion (\(2n = 53\)) or lacking it (\(2n = 54\)). There were no *defassa* group individuals that were homozygous for the 6;18 fusion (\(2n = 52\)), but this may be a sampling artifact. The 6;18 fusion was fixed in the *ellipsiprymnus* group, whereas the 7;11 fusion was absent in the *defassa* group. In G- and C-banded karyotypes, all autosomal arms and the X chromosomes of the two subspecies groups appeared to be completely homologous. However, the Y chromosome was acrocentric in the *ellipsiprymnus* group and submetacentric in the *defassa* group; possibly the result of a pericentric inversion. Fixed chromosomal differences between the two subspecies groups reflect a period of supposed geographic isolation during which time they diverged genetically and phenotypically, and the centric fusion polymorphisms raise the possibility of reduced fertility in hybrids. These data, in conjunction with phenotypic and mitochondrial DNA data, suggest to us that populations of the *ellipsiprymnus* and *defassa* groups should be managed separately.

Chromosomal evolution in the mammalian family Bovidae is believed to have been predominated by centric (Robersonian) fusions. For example, the diploid number of 48 species of bovids varies widely (\(2n = 30-60\)), but the fundamental number is relatively constant (FN = 58–62) in all but three of these taxa (Wurster and Benirschke 1968). Among species representing four of the five subfamilies of Bovidae, banded karyotypes demonstrate that chromosome-arm homologies are extensive, and that shared homologous bi-armed chromosomes are rare, further indicating the importance of centric fusions in bovid chromosomal evolution (Buckland and Evans 1978; Bunch and Nadler 1980; Gallagher and Womack 1992). Monobrachial homologous biarmed chromosomes also distinguish karyotypes within certain bovid genera, notably Damalisus (Kumamoto et al. 1997) and Gazella (Effron et al. 1976; Kumamoto et al. 1995; Vassart et al. 1995).

The degree to which centric fusions have resulted in monobrachial homologies, particularly between closely related species, raises the possibility that speciation (i.e., reproductive isolation) in bovids has occurred by monobrachial centric fusions, a model advanced by Baker and Bickham (1986). A prerequisite to the establishment of monobrachial homologous biarmed chromosomes between populations is the fixation of single centric fusions within populations. It is generally accepted that heterozygotes for single centric fusions often encounter minimal meiotic problems because trivalents are able to segregate normally (Baker and Bickham 1986), and among bovids this has been demonstrated in goitered gazelles (Kingswood et al. 1994). Thus it is not surprising that a number of bovid taxa are polymorphic for centric fusions, including African buffalo (Buckland and Evans 1978), Arabian oryx (Cribiu et al. 1990), Guenther’s dik-dik (Ryder et al. 1989), blackbuck (Effron et al. 1976), impala (Wallace 1980), and several species of gazelles (Arroyo Nombela et al. 1990; Benirschke et al. 1984; Kingswood and Kumamoto 1988; Kumamoto et al. 1995).

In this article we document centric fusion polymorphisms in another bovid, the waterbuck (*Kobus ellipsiprymnus*). Taxonomically, *K. ellipsiprymnus* is comprised of two subspecies groups, the *ellipsiprymnus* group (common waterbuck) and the *defassa* group (*defassa* waterbuck). Ansell (1971) lists four subspecies in the *ellipsiprymnus* group and nine in the *defassa* group, but within these two groups the validity and distribution of many subspecies are not well established. Previously, chromosomes of waterbuck have been reported as \(2n = 50\) (Gallagher and Womack 1992; Wallace 1980; Wurster and Benirschke 1968). The purpose of this study is to describe G- and C-banded karyotypes of common and defassa waterbuck, each subspecies group being polymorphic for one of two centric fusions.

**Materials and Methods**

We studied the chromosomes of 49 specimens of *K. ellipsiprymnus* from five zoos and three individuals from Lake Nakuru.
Figure 1. G-banded karyotypes of *K. ellipsiprymnus*: (a) karyotype of a female (2n = 51) from the *ellipsiprymnus* subspecies group with a boxed inset showing the G-banded sex chromosomes of a male; (b) karyotype of a male (2n = 54) from the *defassa* subspecies group. In (a) the polymorphic centric fusion (7;11) is in parenthesis to distinguish it from fixed fusions. Arrowheads indicate centromeric positions.

Table 1. Summary of chromosomal data for *Kobus ellipsiprymnus*

<table>
<thead>
<tr>
<th>Subspecies group</th>
<th>N</th>
<th>2n</th>
<th>FN</th>
<th>Autosomes</th>
<th>Centric fusions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>m/s</td>
<td>6;18</td>
</tr>
<tr>
<td><em>ellipsiprymnus</em></td>
<td>4:2</td>
<td>50</td>
<td>61–62</td>
<td>10 38</td>
<td>lm</td>
</tr>
<tr>
<td></td>
<td>9:6</td>
<td>51</td>
<td>61–62</td>
<td>9  40</td>
<td>lm</td>
</tr>
<tr>
<td><em>defassa</em></td>
<td>2:6</td>
<td>53</td>
<td>62</td>
<td>8  42</td>
<td>lm</td>
</tr>
</tbody>
</table>

N = sample size (females: males); 2n = diploid number; FN = fundamental number; m/s = metacentric/submetacentric; a = acrocentric; lm = large metacentric; sa = small acrocentric; ss = small submetacentric; ++ = fusion homozygote; +– = fusion heterozygote; – = lacking fusion.

National Park, Kenya; these included 16 females and 10 males of common waterbuck and 10 females and 16 males of defassa waterbuck. Origins of the zoo animals were unknown, but differences between the rump coloration of common and defassa waterbuck (see Kingdon 1982) made it possible to phenotypically identify the subspecies group of each specimen. Metaphase chromosomes were obtained from cell cultures derived either from skin biopsies (ca. 5 mm²) or lymphocytes from 5–10 ml of heparinized whole blood.

For short-term lymphocyte culture, we followed a modified technique of Moorhead et al. (1960) and Wiley and Meisner (1984) using pokeweed mitogen (0.3 ml) and comitogen phorbol 12-myristate 13-acetate-40-methyl ether (final concentration 6 μg/ml). Blood cultures were harvested after a 94 h incubation period followed by a 1 h exposure to colcemid (final concentration 0.025 μg/ml). For fibroblast culture of skin biopsies we used a collagenase disaggregation technique, harvesting cells according to the general protocol for monolayer cultures (Barch 1991). At peak mitotic activity, monolayer cultures were exposed to colcemid (final concentration 0.025 μg/ml) for 10–30 min, and cultures were then exposed to 0.075 M KCl for 10 min prior to fixation of cells.

From the mitotic cell harvests we studied the chromosomes of all 52 animals by nondifferential Giemsa staining. In addition, 30 of the specimens were G-banded using the method of Verma and Babu (1989), and 24 were C-banded following the method of Sumner (1972). Because of the difficulty in comparing G-band homologies between taxa without a standardized nomenclature, G-banded chromosomes were numbered according to the standard karyotype of cattle (*Bos taurus*) presented by the Reading Conference (1980) and Ianuzzi and Di Meo (1995). Gallagher and Womack (1992) demonstrated extensive arm homologies among several species of bovids using the cattle standard. Because waterbuck chromosomes could be identified to cattle equivalents, we followed this convention to facilitate comparisons between our specimens.

**Results**

Chromosomal complements were 2n = 50–52 (FN = 61–62) in the *ellipsiprymnus* group and 2n = 53–54 (FN = 62) in the *defassa* group of *K. ellipsiprymnus* (Figure 1; Table 1). Pericentromeric heterochromatin was pronounced on all acrocentric...
autosomes, but heterochromatic staining of metacentrics was faint and restricted to the centromeric region (Figure 2). In both subspecies groups the Y chromosome and short arm of the X chromosome appeared to stain entirely heterochromatic; Xp was G-band negative, corresponding to the area that was C-band positive.

Variation in the diploid number of K. ellipsiprymnus was the result of polymorphism for two independent centric fusions (Figure 3; Table 1). The ellipsiprymnus group was polymorphic for a 7;11 centric fusion. Both elements of chromosome pairs 7 and 11 were fused in fusion homozygotes (2n = 50); in fusion heterozygotes (2n = 51), only one element of each pair was fused. The 7;11 fusion was lacking in ellipsiprymnus group specimens with 2n = 52 and in all defassa group specimens. The defassa group was polymorphic for a 6;18 centric fusion; individuals were either heterozygous for the fusion (2n = 53) or lacking it (2n = 54). There were no waterbuck of the defassa group that were homozygous for the 6;18 fusion (2n = 52). However, this possibly reflects a sampling artifact since selection against the homozygous form of the fusion seems unlikely. The 6;18 fusion was fixed in all specimens of the ellipsiprymnus group.

It is worth noting that the 6;18 polymorphism was found in the one wild population of waterbuck that we studied. Among the three specimens of defassa waterbuck from Lake Nakuru National Park, one male was heterozygous for the 6;18 fusion (2n = 53), but the fusion was absent in the other two males (2n = 54). However, the extent to which the 6;18 and 7;11 polymorphisms occur naturally is unknown because the captive populations of waterbuck that we sampled may represent a mixture of individuals from different natural populations (Jones ML, in literature). We cannot rule out the possibility that the common waterbuck that were heterozygous for the 7;11 fusion (2n = 51) were the result of captive breeding between animals taken from two natural populations, for example, one population where individuals were homozygous for the 7;11 fusion (2n = 50) and the other where individuals did not have the fusion (2n = 52).

In G- and C-banded karyotypes, all autosomal arms and the X chromosomes of common and defassa waterbuck appeared to be completely homologous. The Y chromosomes of the two subspecies groups were of similar size, suggesting that their morphological differences (see Table 1) were not due to short-arm additions or deletions. The Y chromosomes may differ by a pericentric inversion; however, it is difficult to interpret the G-banding patterns of these small elements. All autosomal arms of both groups of K. ellipsiprymnus could be identified with Bos homologues,

but the pericentromeric banding patterns of chromosomes 9 and 14 of K. ellipsiprymnus were more similar to those of Capra hircus and Ovis aries (see Iannuzzi and Di Meo 1995). The sex chromosomes of K. ellipsiprymnus and B. taurus differed; the cattle X was C-band negative, but the waterbuck Xp was entirely heterochromatic. Xq of K. ellipsiprymnus appeared homologous to theacrocentric X of C. hircus and O. aries, which was reported to differ from B. taurus by para- and pericentric inversions (see Iannuzzi and Di Meo 1995). The Y chromosomes of cattle and defassa waterbuck were similar in morphology (i.e., both were small submetacentrics) and both were G-band positive at the distal end of Yp. However, G-bands of the q arms differed; cattle Yq was entirely G-band positive (see Iannuzzi and Di Meo 1995), but in defassa waterbuck, Yq was G-band negative around the centromere.

Discussion

Chromosomal complements of 2n = 50–54 (FN = 61–62) for K. ellipsiprymnus found in this study compare with previous reports of 2n = 50 (FN = 61–62) for the species (Gallagher and Womack 1992; Wallace 1980; Wurster and Benirschke 1968). Each of these previous studies were based on sample sizes of only one or two specimens. Wallace (1980) studied ellipsiprymnus group waterbuck from a natural population in Kruger National Park, South Africa, but the other studies involved captive populations. Chromosome-arm banding patterns of G-banded karyotypes (Figure 1) correspond with those of the QFH-band karyotype in Gallagher and Womack (1992: Figure 8), including waterbuck chromosomes 9 and 14, which are banded like the chamois, Rupicapra rupicapra (Gallagher and Womack 1992: Figure 5). As in our waterbuck which had complements of 2n = 50, the Gallagher and Womack (1992) waterbuck had five metacentric pairs of autosomes: 1;19, 2;29, 5;17, 6;18, and 7;11. Wallace (1980) and Wurster and Benirschke (1968) did not present karyotypes of K. ellipsiprymnus, but for 2n = 50 waterbuck, their data were consistent with this study.

Karyotypic differences between the ellipsiprymnus and defassa groups of K. ellipsiprymnus reflect differences in pelage coloration and nucleic acid sequences that are believed to have evolved during their geographic isolation (Kat 1993). Waterbuck are found in savannas throughout much of sub-Saharan Africa, but their dis-
Figure 3. G-banded preparations of autosomes involved in the 6;18 and 7;11 fusion polymorphisms of K. ellipsiprymnus: (a) ellipsiprymnus subspecies group (2n = 50–52); (b) defassa subspecies group (2n = 53–54). Arrows indicate centromeric positions.

tribution can be rather localized owing to their preference for mesic habitats (Ansell 1971). For the most part, common waterbuck occur east of the Gregory (eastern) Rift Valley and defassa waterbuck are found west of the Albertine (western) Rift Valley. These two forms are distinguished by a difference in rump coloration; typically, common waterbuck have an elliptical white ring on a dark rump, and defassa waterbuck lack the ring because of their white rump (Kat 1993; Kingdon 1982). This difference was the basis for classification of the defassa group as a separate species, K. defassa (Ellerman et al. 1953). Based on sequence variation in the mitochondrial DNA control region, common and defassa waterbuck populations in Kenya are genetically different from each other (Kat 1993). However, the two groups are sympatric in areas of Kenya and Tanzania, probably as a result of secondary contact. Intergradation of the rump pattern and relationships based on genetic distances (i.e., nucleic acid sequence differences) among populations indicate that common and defassa waterbuck hybridize, and they are now considered conspecific (Ansell 1971; Kat 1993; Kingdon 1982). These two subspecies groups have also hybridized in captivity, and at least one individual lived several years (Gray 1972), but reproduction by hybrids has not been reported.

How is reproduction affected by the chromosomal differences between the ellipsiprymnus and defassa groups of K. ellipsiprymnus? Karyotypes of common and defassa waterbuck (2n = 50–52 and 53–54, respectively) vary largely because of two centric fusions (6;18 and 7;11), each rearrangement being polymorphic in either one subspecies group or the other. The 6;18 fusion, which is polymorphic in defassa waterbuck, is fixed in common waterbuck. The 7;11 fusion is polymorphic in common waterbuck, but this fusion is completely absent in defassa waterbuck. These differences and those involving the Y chromosomes indicate a period during which there was no gene flow between the two groups. However, putative zones of secondary contact and hybridization (see Kat 1993) suggest that common and defassa waterbuck were not separated for a period sufficient to result in reproductive isolation. In a captive population of Soemmering’s gazelles, polymorphisms for three centric fusions were suspected as being related to poor reproduction (Benirschke et al. 1984). Although it is speculative to infer possible segregational difficulties from other bovid taxa, the centric fusion polymorphisms distinguishing common and defassa waterbuck theoretically could lead to reduced fertility in their hybrids or aneuploid offspring.

In conclusion we believe it is appropriate to consider the cytogenetics of waterbuck in the management of their natural and captive populations. The importance of cytogenetics in wildlife management and conservation has been advanced by Benirschke and Kumamoto (1991) and Robinson and Elder (1993). If wildlife conservation is not simply directed toward conservation of species, but also toward the preservation of unique populations, then chromosomal data in conjunction with phenotypic and mitochondrial DNA data suggest to us that populations of common and defassa waterbuck should be managed separately. However, in order to address the conservation of waterbuck more effectively, future genetic research should be directed toward natural populations of this antelope throughout Africa.
Head Spot and Dilute Mutations in the Norwegian Rat

R. Robinson

A mutant allele of a new white spotting locus in the Norwegian rat is described, which is designated as head spot (hs). The allele is inherited as a recessive to normal. The expression is regularly manifested and has the form of a white spot of variable size in the middle of the forehead just above the eyes. A probable reoccurrence of blue dilution coat color is also reported. Tests for genetic linkage for hs indicate that it is independent of the agouti, dilute, and hooded loci.

A white spotting locus, H, has been known for the Norwegian rat since the earliest days of mammalian genetics and is featured prominently in the classic selection experiments of W. E. Castle (see summary in 1951). The locus has been designated hooded after its best known mutant allele (h). The allele produces a characteristic colored head and white body, with a colored spinal stripe that is often, if not usually, incomplete from head to tail. The normal gene is incompletely dominant since the heterozygote Hh typically shows a variable white patch on the stomach. A second allele for the locus is known as Irish spotting (h*), which induces a white spot or an inverted V blaze on the forehead and variable amounts of white on the stomach. The order of dominance is \( H > h > h^* \), although this is usually incomplete. In particular, the heterozygote Hh can be phenotypically similar to \( h^* \).

It should be noted that it is not uncommon for individual rats to possess a variable white patch on the stomach independent of the hooded alleles. This appears to arise by chance ineffective migration of melanoblasts from the neural crest. The size of the patch can be readily increased by selection and is probably mediated by polygenes. The \( h^* \) allele can increase the size of the patch, while the \( h \) allele produces a fully white stomach.

The present blue mutant rat was discovered in a shop that sells rodents as pets by a fancier who was sufficiently knowledgeable to realize that the color was novel. The coat is slate blue and agrees closely with the description given by Roberts (1929) for a previously discovered blue dilution mutant.

Materials and Results

The head spot trait was observed as a segregant from a stock of agouti animals that were of wild origin (Greaves 1981; Robinson 1988). Interbreeding of the mutant showed that head spot was consistently expressed. The size of the spot was variable, ranging from a small spot of a few hairs to roughly 1 cm in diameter. Over 200 individuals were examined. The stomach frequently had a variable white patch that did not differ in size from belly spots commonly found in rats.

To determine the mode of inheritance of the head spot, agouti spot rats were mated to nonagouti (black) rats. The F1 prog-