Hybridization can occur naturally among diverging lineages as part of the evolutionary process leading to complete reproductive isolation, or it can result from range shifts and habitat alteration through global warming and/or other anthropogenic influences. Here we report a molecular cytogenetic investigation of hybridization between taxonomically distinct species of the Alcelaphini (Alcelaphus buselaphus $2n = 40$ / Damaliscus lunatus $2n = 36$) and the Tragelaphini (Tragelaphus strepsiceros $2n = 31/32$ / Tragelaphus angasii $2n = 55/56$). Cross-species fluorescence in situ hybridization provides unequivocal evidence of the scale of karyotypic difference distinguishing parental species. The findings suggest that although hybrid meiosis of the former cross would necessitate the formation of a chain of seven, a ring of four and one trivalent, the progeny follow Haldane’s rule showing F1 male sterility and female fertility. The tragelaphine F1 hybrid, a male, was similarly sterile and, given the 11 trivalents and chain of five anticipated in its meiosis, not unexpectedly so. We discuss these findings within the context of the broader evolutionary significance of hybridization in African antelope, and reflect on what these hold for our views of antelope species and their conservation.
Table 1. Chromosomal composition of alcelaphine and tragelaphine species pairs and their F1 hybrids, as identified by G-banding and confirmed by comparative FISH. Chromosomes are numbered according to the cattle standard [7].

<table>
<thead>
<tr>
<th>parental species</th>
<th>chromosomal composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>(haploid chromosome number)</td>
<td>F1 hybrids</td>
</tr>
<tr>
<td><strong>tribe Alcelaphini</strong></td>
<td>alcelaphine F1 hybrid (2n = 38; see figure S1)</td>
</tr>
<tr>
<td><em>Alcelaphus buselaphus</em> (20,Y): 1;10, 2;25, 3;19, 4;6, 5;14, 7;9, 8;17,</td>
<td>1;10, 1;10, 2;25, 2;25, 3;19, 3;19, 4;14, 4;6, 5;14, 5;14, 7;9, 7;9, 8;17, 8;17, 11;15, 11;23, 12;16, 12;16, 13;15, 13, 14, 14, 14, 18, 18, 18, 18, 20, 20, 20, 20, 20, 20, 20, 21, 21, 21, 22, 22, 26, 26,</td>
</tr>
<tr>
<td>11;15, 12;16, 22;23, 13, 18, 20, 21, 24, 26, 27, 28, 29, Y</td>
<td>27, 27, 28, 28, 29, 29, X; Y</td>
</tr>
<tr>
<td><strong>tribe Tragelaphini</strong></td>
<td>tragelaphine F1 hybrid (2n = 43; see figure S2)</td>
</tr>
<tr>
<td><em>Tragelaphus strepsiceros</em> (15,t(Y;13)): 1;29, 3;10, 4;5, 6;20, 7;18, 8;17, 9;27, 11;23, 12;16, 14;26, 15;28, 19;21, 24;22, 25, Y;13</td>
<td>11;22,22, 24;22, 24, 1;29, 1, 29, 3;10, 3, 10, 4;5, 4, 5, 6;20, 6, 20, 7, 18, 7, 18, 8, 17, 8, 17, 9;27, 9, 27, 11;23, 23, 12;16, 12, 16, 14;26, 14, 26, 15;28, 15, 28,</td>
</tr>
<tr>
<td>11;22,22, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28,</td>
<td>19;21, 19, 21, 25, 25, X; Y;13, 13</td>
</tr>
<tr>
<td>29, X</td>
<td></td>
</tr>
</tbody>
</table>

The second, a *Tragelaphus strepsiceros* (greater kudu) × *Tragelaphus angasii* (nyala) F1 male arose from hybridization on a game farm in the North West Province of South Africa [6]. We report the outcome of a molecular cytogenetic investigation of these hybrid animals to raise awareness of the broader evolutionary significance of hybridization in African antelope, the variability in evolutionary time distinguishing hybridizing species pairs and the implications this holds for antelope diversity and conservation.

2. Material and methods

Tissues collected for the study were derived from adult male F1 hybrids. The culture of fibroblasts, G-banding of chromosomes in situ (FISH) followed established protocols. Cross-species FISH, using cattle (*Bos taurus; BTA*) whole chromosome painting probes, was conducted to confirm (and in some instances correct) chromosomal homology among parental taxa as well as their hybrids (electronic supplementary material). Chromosomes are numbered according to cattle standard [7].

3. Results

(a) Cytogenetics of the *Alcelaphus buselaphus × Damaliscus lunatus* F1

The G-banded karyotypes of both parental species have previously been published and are consequently not presented (electronic supplementary material, table S1). The red hartebeest (*A. buselaphus*) has 2n = 40 and the tsessebe (*D. lunatus*) 2n = 36. Meiosis in male red hartebeest would yield gametes with 20,Y and female tsessebe gametes with 18,X; the presumptive F1 hybrid with 2n = 38 is consistent with this (table 1; electronic supplementary material, table S1). FISH analysis of fusions characterizing the parental species provides unequivocal identification that chromosomal rearrangements in the parental species are present in the F1 hybrid (figure 2).

(b) Cytogenetics of the *Tragelaphus strepsiceros × Tragelaphus angasii* F1

Banded karyotypes have been published for both Tragelaphini parental species and are not repeated (electronic supplementary material, table S1). The greater kudu (*T. strepsiceros*) has 2n = 31/32 and meiosis in males would yield 15,t(Y;13) gametes and, in the female nyala (*T. angasii*) (2n = 56,XX), gametes with 28,X—both consistent with the 2n = 43 complement observed in the F1 hybrid male (table 1; electronic supplementary material, figure S2). FISH analysis of fusions characterizing the parental species provides unequivocal identification that chromosomal rearrangements in the parental species are present in the F1 hybrid (figure 2).

As with the alcelaphine hybrid, the greater kudu × nyala cross was considered sterile following detailed clinical and reproductive potential assessments [6]. This may be anticipated given the 11 trivalents (4–4.5–5; 3–3.10–10; 1–1.29–29; 6–6.20–20; 7–7.18–18; 8–8.17–18; 12–12.16–
Figure 1. FISH using cattle (BTA) chromosome painting probes to validate some of the brachial combinations resulting from Robertsonian (Rb) fusions in the chromosomal complement of the F1 hybrid of a cross between a red hartebeest male and a female tsessebe. (a) Rb4;6, (b) Rb5;14, (c) Rb5;6 and (d) Rb4;14.

Figure 2. FISH using cattle (BTA) chromosome painting probes to validate some of the brachial combinations resulting from Robertsonian (Rb) fusions in the chromosomal complement of the F1 hybrid of a cross between a greater kudu male and a female nyala. (a) Rb2;22;11 and Rb2;22;24, (b) Rb3;10, (c) Rb11;23 and (d) Rb6;20.
6.1–7.3 Ma for last shared common ancestry in fairly deep evolutionary time—definitions (such as the phylogenetic species concept) that view (the biological species concept or BSC) to new species definitions (including game farms), where the morphological distinctiveness of hybrids is relatively subtle (see for example the electronic supplementary material, figure S3), introgression (electronic supplementary material, figure S4) among species/taxa (possibly leading to adaptive introgression, [4]), there is considerable temporal variation in the likelihood that should hybridization be successful, reproductive impairment will result. These considerations add to the complexity of formulating policy to manage biota in changing landscapes, particularly where increased contact between ecologically segregated species may result, or historic distributions and species habits are manipulated or ignored. They are also a challenge to agencies whose conservation policies are largely founded on the biological species concept (reproductive isolation).

Ethics. Samples were collected by veterinarians of the Free State Department of Economic Development, Tourism and Environmental Affairs, and the National Zoological Gardens of South Africa in accordance with the guidelines of the South African Veterinary Council (SAVC) and processed under Stellenbosch University’s ethics no. SU-AUD15–00103.

Data accessibility. Electronic supplementary information supporting this manuscript has been uploaded.

Authors’ contribution. T.J.R. conceptualized the study and drafted the paper with input from H.C. and A.D. (laboratory work) and

4. Discussion

Although both instances of hybridization reflect anthropogenic influence (controlled access to conspecifics in the red hartebeest × tsessebe cross, low densities of species pairs in the case of the greater kudu × nyala cross; details in the electronic supplementary material), it is not known how frequently these occur in nature and whether the incidence has been heightened through range extension (due to translocation, principally through game farming and/or habitat modification). It is noteworthy, however, that the historic ranges of the red hartebeest and tsessebe overlapped naturally north of South Africa, and in areas to the north of the Orange River in the northwestern parts of the Free State Province, South Africa [12,13]. The greater kudu and nyala show extensive sympatry in the southeastern parts of the southern African subregion [14]. Given these distribution patterns, it seems that although hybridization can occur between these taxa, the genetic integrity of the species pairs (through introgression of genetic and phenotypic characters from one species into the other) is not significantly compromised, even if female-mediated gene flow seems possible in the case of red hartebeest × tsessebe (electronic supplementary material, figure S5). Distinctiveness is likely underpinned by assortative mating and habitat specificity (D. lunatus prefers fringes of grassland that merge into woodlands, while A. buselaphus occurs in open areas and avoids closed woodland [14]). Interestingly, there is one recorded instance of a red hartebeest × tsessebe hybrid observed in a natural setting [5] that predates anthropogenic influence, suggesting that hybridization is insufficient in nature to homogenize the nuclear gene pool, and that progeny of mixed parent pairs probably have reduced fitness under competitive conditions.

In the broader sense, however, these data serve to highlight several important considerations associated with the radiation of many of the African antelope. Using a recently published multi-calibrated molecular phylogeny to broadly affix divergence times of hybridizing species pairs, it would appear that the viable F1 offspring detected here result from crosses between species that last shared common ancestry in fairly deep evolutionary time—6.1–7.3 Ma for T. strepsiceros and T. angasi and 3.2–5.1 Ma for Damalisus (proxy for D. lunatus) and A. buselaphus (see fig. 1 in [2] for 95% range for node ages). There are other documented instances of hybridization in both tribes. In the Tragelaphini: Tragelaphus scriptus × Tragelaphus (Taurotragus) oryx (4.5–5.4 Ma; 6.1–7.3 Ma), Tragelaphus speki × Tragelaphus (Booceros) euryceros (3.0–3.6 Ma) and T. speki × Tragelaphus imberbis (6.8–8.0 Ma). Hybrid offspring were recorded from all three crosses (a fertile female hybrid in the case of T. speki × T. euryceros; [1]). In the Alcelaphini, hybrids have been recorded between Connochaetes taurinus × Connochaetes gnou ([15]; 0.7–1.6 Ma) and A. buselaphus × Pygargus phillipsi phillipsi ([16]; 3.2–5.1 Ma). F1 hybrids are fertile in the former and sterile in the latter cross.

In the relatively recent past, there has been a move away from defining species on grounds of reproductive isolation (the biological species concept or BSC) to new species definitions (such as the phylogenetic species concept) that view species as a continuum of separately evolving lineages that extend from ecological races to hybridizing species and, ultimately, to species that no longer cross [17,18] (see [19] for discussion in ungulates). In other words, there is a loss of the tendency to hybridize that is almost clock-like and, as a consequence, much of the evolution leading to reproductive isolation occurs while gene flow persists [20]. In fact, there is considerable evidence to suggest that hybridization, which is individually rare, is relatively frequent in plants (25%) but less so in animal species (10%). Six per cent of European mammal species are thought to hybridize [20]. It is important therefore to understand the context in which hybridization occurs and, where possible (and/or necessary), manage the consequences appropriately.

We anticipate that hybridization, possibly with attendant hybrid zones [21], may be detected in some antelope. This would be in keeping with hybridization being a natural phenomenon in their evolutionary history, a process that is thought generally to have led to the persistence of species through periods of climate change [3]. African antelope likely to exhibit hybridization are those where population structure depends on geographical distance moulded by fragmented habitats (particularly forests, grassland patches and wetlands that are subjected to repeated interglacial cycles of expansion and contraction [22–24]). These include Redunucini (waterbuck and allies, particularly within Redunca 3.4–5.2 Ma and Kobus 2.4–3.4 Ma), plains antelope such as the Antilopini (gazelles, particularly in Gazella: 2.3–3.1 Ma), forest taxa such as the Cephalophini (duikers, particularly in Cephalophus: 5.6–7.2 Ma) and among Hippotraginari (4.6–6.7 Ma [25], 1.6–2.4 Ma [1]). There are recorded [1,15,16,25] and recent instances of hybridization (electronic supplementary material, figure S4) among species of all the tribes indicated above. Although this is clearly not likely to lead to introgression in all instances, these data are, nonetheless, of particular ex situ conservation concern. In more natural settings (including game farms), where the morphological distinctiveness of hybrids is relatively subtle (see for example the electronic supplementary material, figure S5), detection is probably underestimated, confounding detailed scrutiny and appropriate interventions, should these be required.

In general, although hybridization is more likely among closely related species/taxa (possibly leading to adaptive introgression, [4]), there is considerable temporal variation in the likelihood that should hybridization be successful, reproductive impairment will result. These considerations add to the complexity of formulating policy to manage biota in changing landscapes, particularly where increased contact between ecologically segregated species may result, or historic distributions and species habits are manipulated or ignored. They are also a challenge to agencies whose conservation policies are largely founded on the biological species concept (reproductive isolation).
E.S. (fieldwork). All authors participated in data analysis, contributed to and approved the content of the final manuscript and acknowledge joint accountability for its content.

Competing interests: We declare we have no competing interests.

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References


