INFO SHEET

1 Mitochondrial testing

Mitochondrial DNA (mtDNA) is inherited through maternal lineages and is equivalent to male surname transmission in humans. The mtDNA test can be used to (a) determine the historical (evolutionary) origin of the family line, *i.e.* to establish the geographical origin of an individual's family line, and (b) trace the number of family lineages in a specific population. This is the only way to determine where the animal's line is (geographically) from.

2 Nuclear testing

Nuclear DNA is inherited through both parents, and offspring relate to each parent by 50%. This test provides information about the individual itself, but is not necessarily very accurate if you wish to know where the animal's line comes from originally. This test is designed to provide information about the purity of an animal. The nuclear test determines the percentage membership of an individual to different geographic groups and gives an individual's nuclear profile ("purity") in percentage.

3 Relatedness/Parentage

This test is used to answer a relatedness question. The client usually has a specific question in mind. For example, is animal A the parent of animal B? Relatedness analyses/parentage is based on the nuclear profile.

4 What are the benefits of testing?

Mitochondrial testing determines the historical origin of an individual. Nuclear testing provide information about the purity of the animal. These two markers therefore provide complementary information about the evolutionary origin of lineages (mitochondrial DNA) as well as migration between/mixing of different populations (nuclear markers). Where no information is available around breeding practice or family lines on farms, mitochondrial DNA can assist to narrow the number of potential parents/siblings (*i.e.* the number of questions).

Therefore, combining these types of tests help breeders to understand the genetic make-up of the herds and will assist (a) farm managers with a particular breeding strategy and (b) prospective buyers in making the best selection for their herds.

5 How to use the results

First, breeders need to decide on a breeding strategy early on. The focus can either be on (a) high levels of genetic diversity and variation, or (b) population purity.

To achieve high levels of diversity and variation, a breeder may want a nuclear result that have membership to some or all of the different groups. The nuclear profile can be combined with mitochondrial membership to any of the genetic groups.

Population purity requires the nuclear result to correspond with mitochondrial membership. In this case, a breeder may want a nuclear result that reflects a single genetic group, *i.e.* 90% Zambian, that is the same as the mitochondrial membership, *i.e.* Zambian or western Zambian.

To achieve a particular breeding strategy, all or the overriding majority of animals in the core breeding group should subscribe to these genetic principles. Importantly, breeders must be aware that offspring may reflect ancestral polymorphisms, or what is also refferd to as "old blood" (diversity in ancestry). The breeding process is unpredictable, and offspring may show unexpected membership to non-selected groups – because of the diversity in the ancestry of animals.

6 Understanding the report

6.1 Sable report

The geographical distribution areas and membership groups are illustrated in **Annexure A**.

The mitochondrial results are presented in table format. **Sable antelope** (*Hippotragus* niger) are grouped into seven geographical distribution areas.

The nuclear results are presented in table format. **Sable antelope** (*Hippotragus* niger) can have a percentage membership to the following groups: (a) Angolan, (b) Zambian, (c) Southern, (d) West Tanzanian, and (e) Eastern. Animals with individual assignment above 90% to one population can be considered as pure animals. All remaining animals can confidently be considered the result of mixing of different populations, the degree of mixing is given as a percentage membership to the different populations. As is standard practice in biological studies, a threshold of 5% is used for significance (*i.e.* results below 5% are not shown).

6.2 Roan report

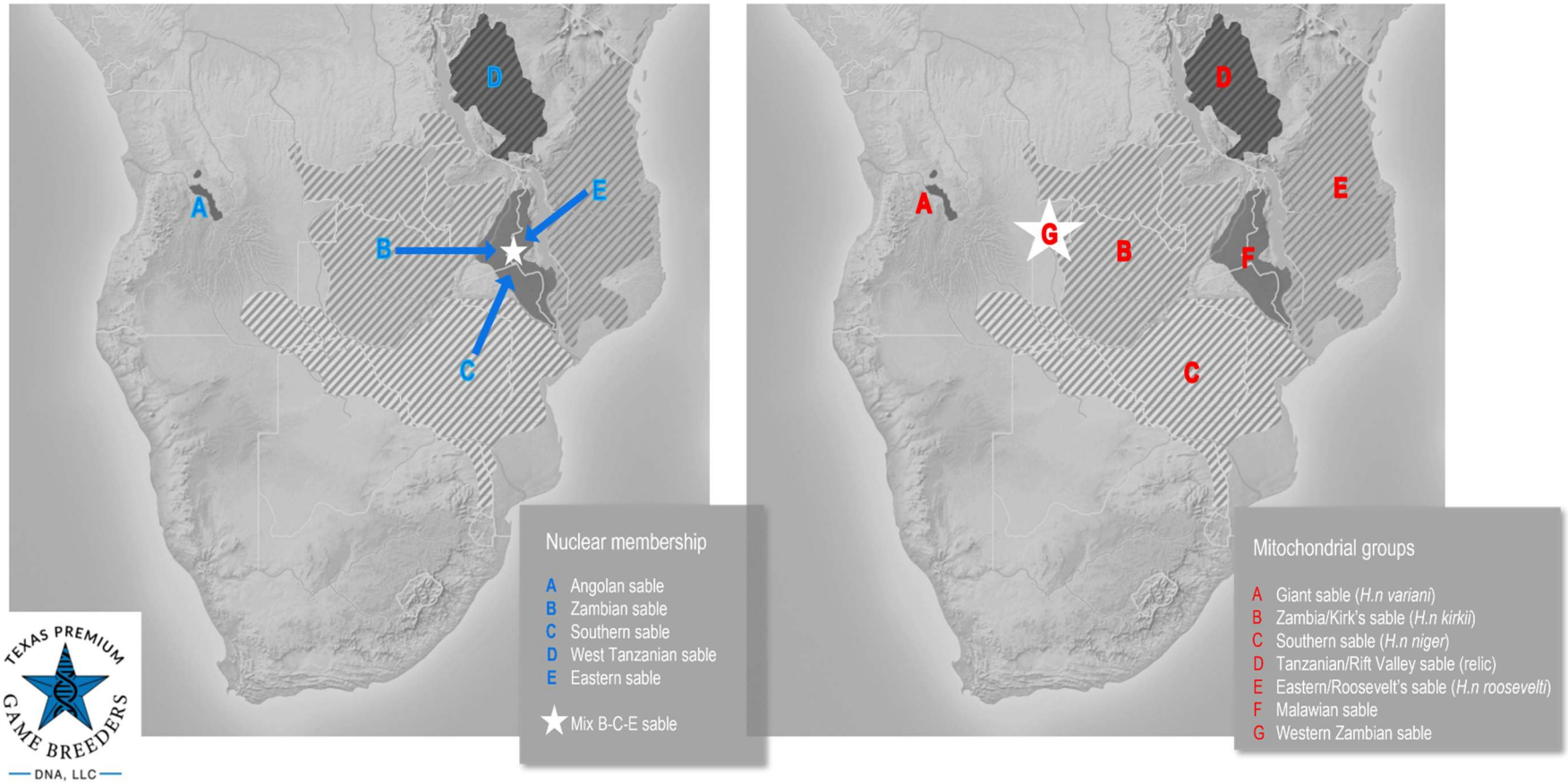
The geographical distribution areas and membership groups are illustrated in **Annexure B**.

The mitochondrial results are presented in table format. **Roan antelope** (*Hippotragus equinus*) are grouped into six geographic distribution areas.

The nuclear results are presented in table format. **Roan antelope** (*Hippotragus equinus*) can have a percentage membership to the following groups: (a) southern African/*equinus*, (b) central African/*cottoni*, (c) east African/*langheldi*, (d) northeastern African/*bakeri*, (e) north central Africa/*charicus*, and (f) northwest Africa/*koba*. Animals with individual assignment above 90% to one population can be considered as pure animals. All remaining animals can confidently be considered the result of mixing of different populations, the degree of mixing is given as a percentage membership to the different populations. As is standard practice in biological studies, a threshold of 5% is used for significance (*i.e.* results below 5% are not shown).

ANNEXURE A

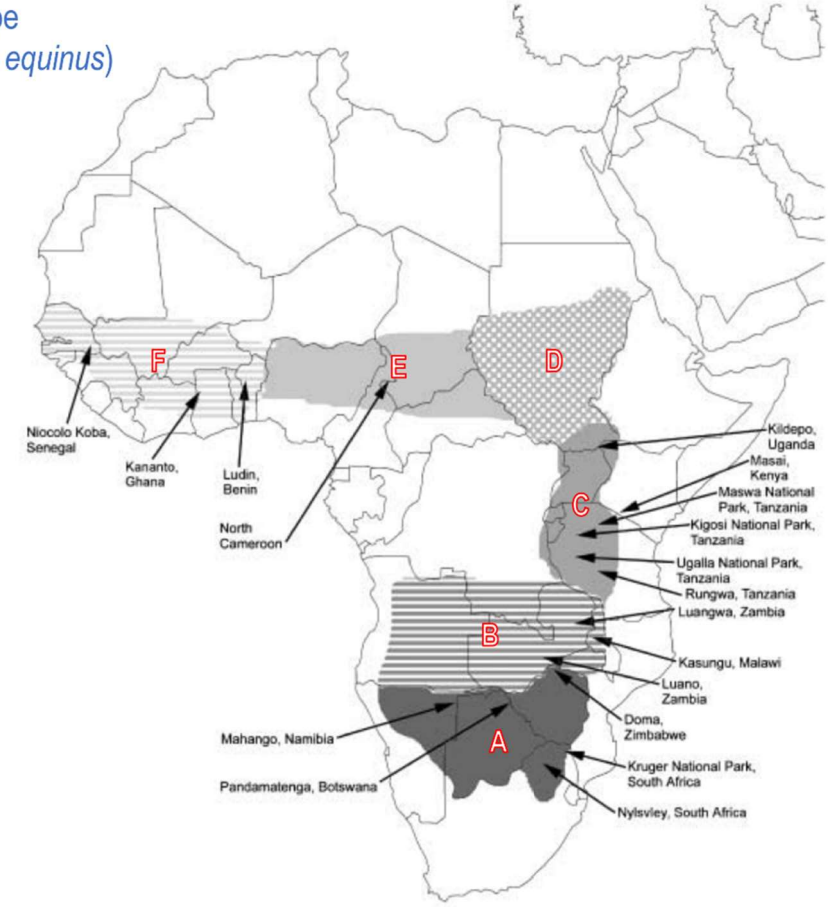
Sable antelope
(*Hippotragus niger*)



TEXAS PREMIUM GAME BREEDERS — DNA, LLC

Adapted from: Jansen van Vuuren *et al.* (2010) *South African Journal of Wildlife Research* 40(1): 35-42; Vaz Pinto *et al.* (2015) *European Journal of Wildlife Research* 61: 313-317.

Roan antelope
(*Hippotragus equinus*)



Mitochondrial groups/Nuclear membership

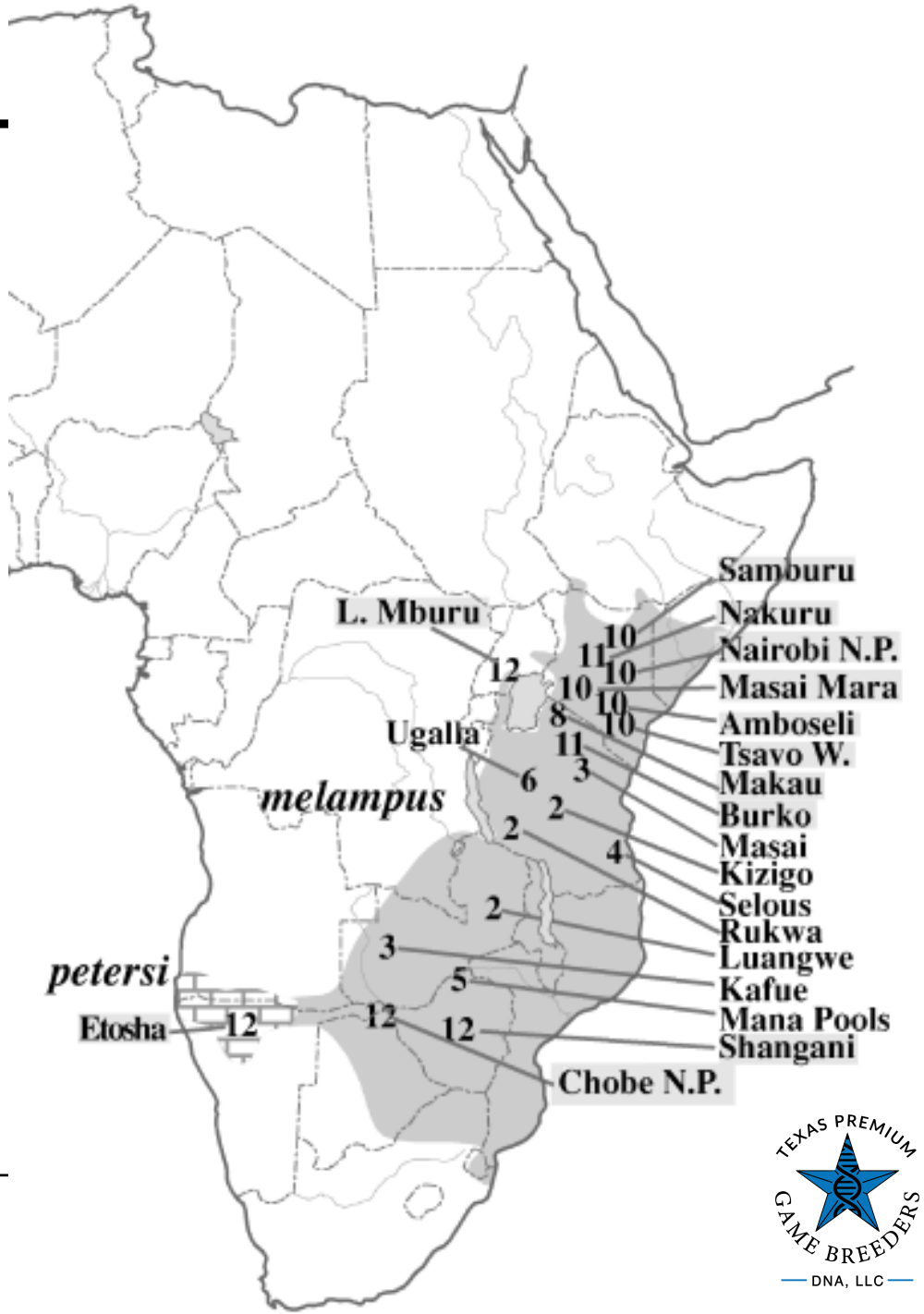
- A southern African/*H.e equinus*
- B central African/*H.e cottoni*
- C east African/*H.e langheldi*
- D northeastern African/*H.e bakeri*
- E north central African/*H.e charicus*
- F northwest African/*H.e koba*

Adapted from: Gonçalves *et al.* (2021) *Journal of Biogeography* 48: 2812-2827; Alpers *et al.* (2004) *Molecular Ecology* 13: 1771-1784.

IMPALA

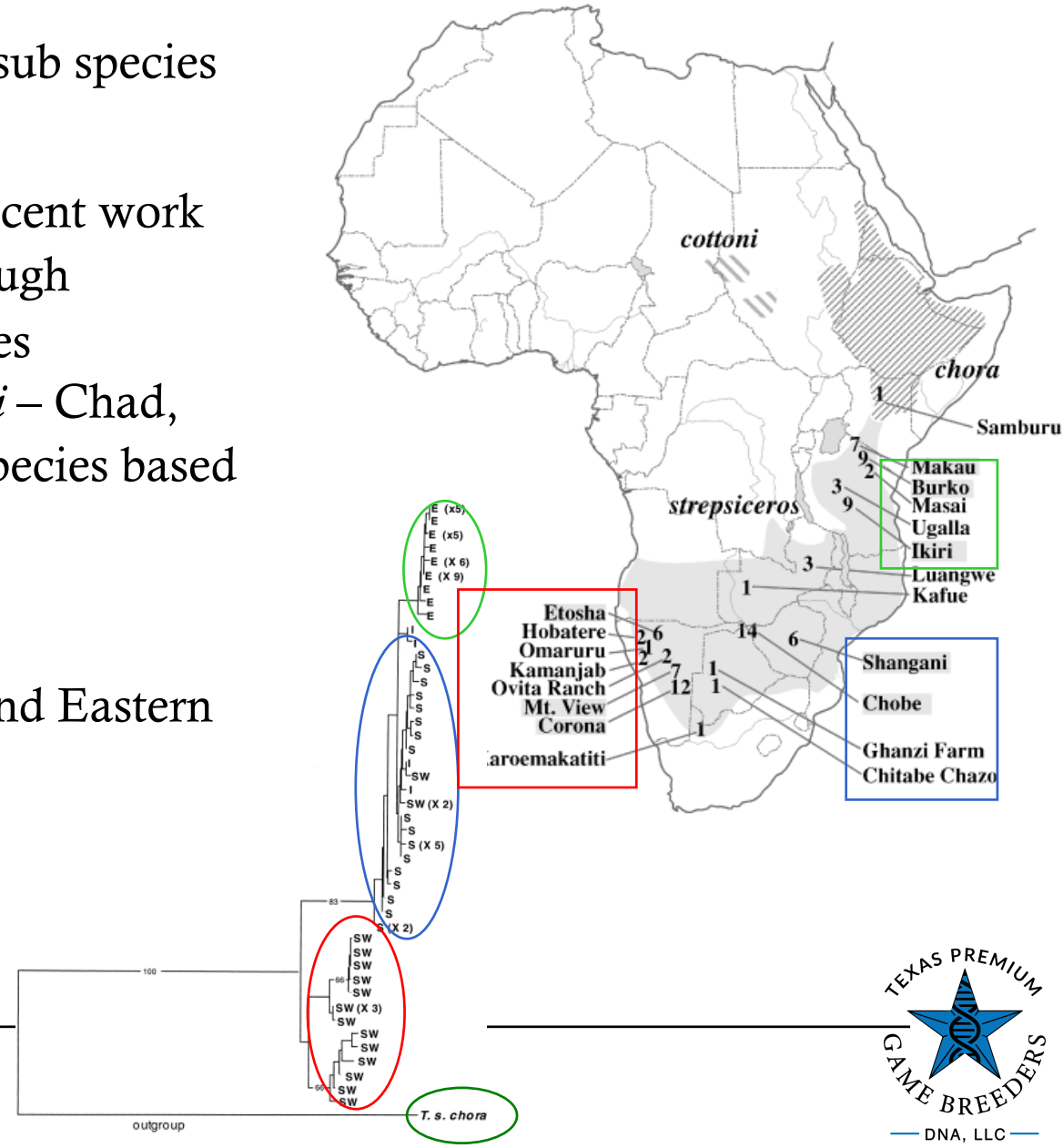
(***Aepyceros melampus)***

- Impala *Aepyceros melampus* – Recognizing two distinct sub-species:
- *A.m.petersi* (Black Faced Impala, 2008 -3000 individuals)
- *A.m.melampus* (Common and widespread - Southern and Eastern Impala)
- 155 samples from 19 localities



GREATER KUDU (Tragelaphus strepsiceros)

- Greater Kudu *Tragelaphus strepsiceros* – Recognizing sub species based on historical regions, color and stripe patterns
- During the past, 4 subspecies were recognized but recent work lists only a single subspecies (e.g. Grubb 1993) although Jonathan Kingdon (1997) still recognizes 3 subspecies (*T.s.strepsiceros* – East and Southern Africa, *T.s.cottoni* – Chad, Western Sudan, *T.s.chora* – North-east Africa). Subspecies based on color and stripe patterns
- 90 samples from 19 localities
- Clear distinction between Southwestern, Southern and Eastern haplotypes



6.3 Relatedness report

Results are given as a percentage probability. Low genetic diversity (inbreeding) influences the assignment probabilities significantly. A 95% probability usually confirms the relationships between individuals, but where groups are highly related, this becomes difficult, and parent-offspring probabilities can decrease to 90%.

7 Further reading

Alpers *et al.* (2004), Population genetics of the roan antelope *(Hippotragus equinus)* with suggestions for conservation. *Molecular Ecology* 13: 1771-1784.

Gonçalves *et al*. (2021), Evolutionary history of the roan antelope across its African range. *Journal of Biogeography* 48: 2812-2827.

Jansen van Vuuren *et al.* (2010), Western Zambian sable: Are they a geographic extension of the giant sable antelope? *South African Journal of Wildlife Research* 40(1): 35-42.

Vaz Pinto et al. (2015), First estimates of genetic diversity for the highly endangered giant sable antelope using a set of 57 microsatellites. *European Journal of Wildlife Research* 61: 313-317.