Parenthood analysis of the African Wild Dog from the Mkomazi Game Reserve in Tanzania

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Material

This report describes a DNA fingerprint analysis of seven blood samples of the African Wild Dog from the Mkomazi Game Reserve in Tanzania.
The collected samples contained:

	Code	Identity
Llondirrigiss	297	mother
Najo	300	presumable father
Kisima	333	pup
Kisima	335	pup
Kisima	336	pup
Kisima	337	pup
Kisima	339	pup

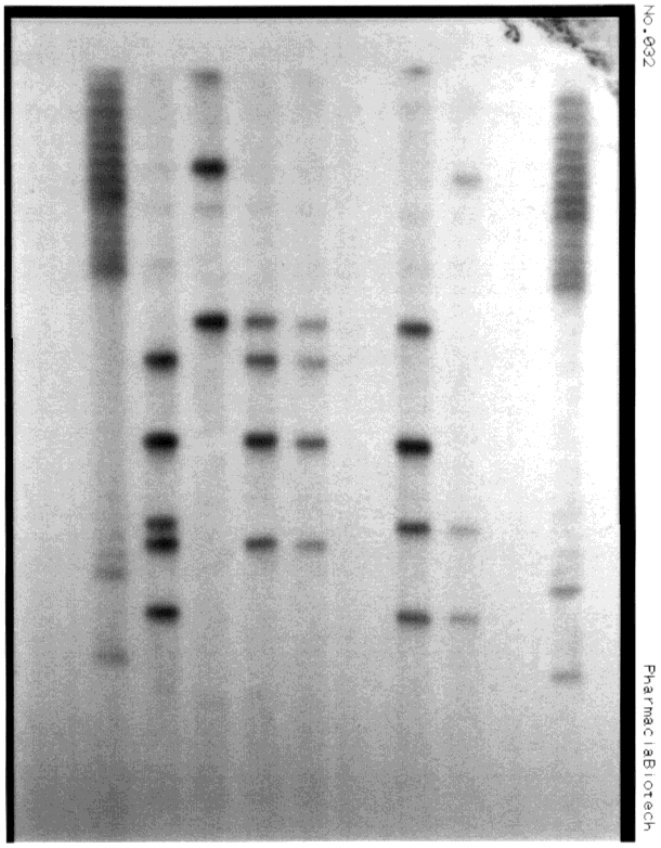
Method

1. DNA isolation

DNA was isolated from 3 ml whole blood collected in EDTA anticoagulant vacutainer tubes. DNA was extracted by proteinase K digestion (100 μg/ml) at 55º C overnight in lysisbuffer (10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 25 mM EDTA, pH 8.0, 0.5% SDS (w/v)), and purified by several extractions with phenol and chloroform:isoamyl-alcohol (24:1).

2. DNA fingerprinting

Five micrograms of DNA were digested to completion with 15 units restriction enzyrn *Hin*fI. DNA fragments were resolved on a 0.8% agarose gel, without ethidium bromide, in a TBE buffer (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA) for 60 h at 1.8 V/cm. After electrophoresis the DNA fragments were transferred to nylon membranes by vacuum blotting and cross linked by UV illumination.
Minisatellite probe 33.15 (Jeffreys) was radioactive labeled with ^{32}P by random-primed DNA labeling. The membrane was hybridized with the radioactive labeled probe and was carried out overnight at 65ºC in hybridization mix
(0.263 M Na_2HPO_4, pH 7.2, 7% SDS (w/v), 1 mM EDTA).
The membrane was exposed to X-ray film for 1 day at -70º C using intensifying screens.



DNA fingerprint of African Wild Dog.
DNA was digested with restriction enzym *Hin*fI
and hybridized with minisatellite probe 33.15.

3. Data analysis

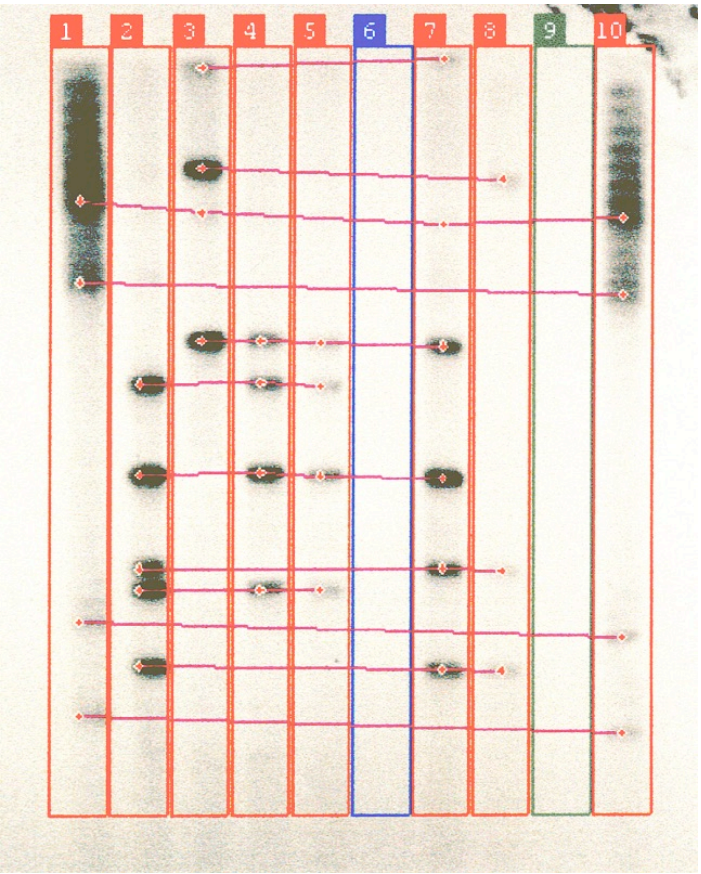
The resulting film with DNA fingerprinting data was analyzed with ImageMaster analyzing equipment. The data were analyzed with DNA Image analysis Elite - ID software.

Results

With the DNA Image analysis software a band matching was executed by computer. This means that bands, detected by the computer and assigned with a  on this picture, that are situated on equal heights, are connected by a purple line.

The lines that are marked between lanes 1 and 10 (marker lanes) are reference lines used to match the other bands of the samples.
The other purple lines are matching lines.

Lane 6 (blue) contained insufficient DNA to detect a signal, so no bands are visible.



Analysis of the DNA fingerprint membrane with use of the matching program software in ImageElite.

Legends of the gel

1. λ marker
2. 297 (mother)
3. 300 father
4. 333 (pup)
5. 335 (pup)
6. 336 (pup)
7. 337 (pup)
8. 339 (pup)
9. negative control
10. λ marker

Conclusion

From the data can be concluded that five bands assigned to the mother (dog 297) are independent of the three bands shown by the father (dog 300), there are no shared bands by mother and father. All descendants show bands that can be assigned to the father or the mother. All bands of the descendants can be assigned to father and mother. From the data can be concluded that dog 300, the presumed father, is the most probable father of the pups 333, 335, 337 and 339 (no results of dog 336 could be found). The possibility of fatherhood of one of the brothers of the mother, dogs 289, 291, 293, 294, 296 or 298, is very unlikely.