Institution: University of Glasgow

Unit of Assessment: Unit 5, Biological Sciences

Title of case study: A genetically informed management plan for breeding African Wild Dogs in captivity

1. Summary of the impact

African Wild Dogs (*Lycaon pictus*; referred to as 'AWDs' hereafter for brevity) have been classed as endangered by the International Union for Conservation of Nature (IUCN) for 22 years. Large, well-managed captive breeding programmes provide a safety net to restore wild populations. However, the management of the AWD population has been difficult owing to an incomplete family record of captive AWDs, which risks introducing genetic disorders caused by inbreeding. A genetically informed management plan developed by University of Glasgow researchers has provided a genetic measure of diversity and establishes a genetically informed pedigree, which is used in the European Endangered Species Programme for African Wild Dogs. This has introduced a more informed means to manage the captive AWD population, to maintain the genetic diversity of the species across the European zoo network (roughly half the world's captive AWD population), with 53 zoos in 16 European countries (and Israel) currently participating.

2. Underpinning research

Keeping captive populations of animals in zoos is often justified on the basis that these populations provide a potential source for the re-introduction of endangered species into the wild. However, there is a risk of losing genetic variation and introducing genetic abnormality into wild populations if individual zoos allow inbreeding within their captive populations. For this reason, zoos maintain a network to exchange animals for breeding. There are many factors that must be considered when selecting animals to breed; this is particularly true for highly endangered African Wild Dogs, which live and breed in packs and operate a breeding hierarchy where typically only the alpha pair breeds. While zoos have focused on ensuring outbreeding, less emphasis has been placed on considering any genetic factors that may limit breeding success or whether captive populations have sufficient levels of genetic variation to be useful as a source for re-introduction.

Between 2006 and 2013, University of Glasgow research led by Dr Barbara Mable at the University of Glasgow investigated the genetic diversity of AWDs within the European zoo network and wild populations in Africa. The original concept, developed by Professor Cleaveland (then at the University of Edinburgh), had been to determine whether genetic diversity of the immune system in AWDs might influence mate-attraction or disease resistance – the aim being to see whether this might improve the bonding of new AWD packs in captive breeding or reduce disease susceptibility. Cleaveland approached Mable, an evolutionary geneticist, who developed the genetic approach to investigate genetic diversity of immune-related genes compared to neutral markers (detailed below). However, the poor zoo records of AWD behavioural data and disease records led instead to a programme in which data on genetic diversity generated by the Glasgow researchers was used to create a management plan that could be used to inform captive breeding across the European zoo network.

The genetic profile of African Wild Dogs

In 2006, Mable developed working partnerships with the Royal Zoological Society of Scotland (Rob Thomas at Edinburgh Zoo, who provided the Glasgow team with contacts at other zoos) and Dr Lorna Kennedy from the University of Manchester (an expert in canid immune gene characterisation). Cleaveland, who joined the University of Glasgow in 2008, continued playing an active role throughout, to liaise with field researchers working with wild AWDs in Africa. The research, which was driven wholly by the University of Glasgow, used a combination of studbook (pedigree) data and analysis of genetic markers of diversity generated from DNA samples collected from the majority of individuals in the European breeding programme, as well as samples provided by field researchers in Africa.[1]

Both 'neutral' and 'adaptive' genetic markers of diversity were analysed. Neutral markers include genes that have no effect on evolutionary fitness and are thus independent of selection by the

environment. By contrast, adaptive markers include genes that are important for adaptation (and thus evolutionary fitness), such as those encoding the major histocompatibility complex (MHC) involved in immune responses.

The combined neutral and adaptive MHC data showed that wild populations maintain surprisingly high levels of genetic diversity despite severe habitat fragmentation and continuing persecution of the AWDs by humans. This high genetic diversity could be due to their extensive home ranges and the hierarchical structure of packs.[1,2] The Glasgow team also showed that the captive populations in the European zoo network maintain approximately 79% of the diversity found in wild populations.[3] However, the research also showed that in some European zoos, extensive inbreeding had been allowed to occur by keeping large numbers of offspring from the same parents, causing a loss of genetic diversity and risking adverse effects of inbreeding. Interestingly, the research also showed that the founders of zoo populations derive from southern Africa, where AWDs are genetically distinct from those in eastern Africa, suggesting that any re-introduction programmes should be restricted to southern populations.[3] In addition, the genetic data demonstrated that some of the AWDs recently imported into zoos, and which were supposed to have come only from southern-Africa-derived captive populations, must have been imported from wild populations in other parts of Africa. This reveals that while introducing new animals to the European zoo network could further increase genetic variation, it comes at the risk of encouraging poaching in wild populations.

Developing a genetically informed management plan

Based on the results of their genetic study,[1,2] the Glasgow team developed a genetic management plan for captive populations that balances genetic priorities with behavioural issues associated with mixing AWD social groups.[3] Key factors in this plan included: (i) the selection of breeding groups that provide equal representation of each of the founder families of the captive population; (ii) limiting individual family sizes by permitting breeding opportunities to no more than two sibling groups (groups of sisters or groups of brothers that are combined for breeding) from the same parents, with breeding pairs limited to producing two litters; (iii) not splitting groups of same-sex relatives unless for breeding, when a new breeding group can be formed by bonding small numbers of males and females; (iv) the continued separate management of captive populations in the USA, Australia, Europe and South Africa (with occasional migration of AWDs between plans) to help maintain variation across the comparatively small captive population (554 across 104 institutions worldwide). The genetic profiling of animals that were born, or introduced, to the European zoo network is on-going at the University of Glasgow, led by Mable. The genetically informed management plan represents a balance that is sound from a genetic perspective and practical for implementation within the European zoo network.

Key University of Glasgow researchers: Dr Barbara Mable (NERC Advanced Research Fellow, 2006–2011; Reader, 2011–present); Professor Sarah Cleaveland (Professor of Comparative Epidemiology, 2008–present).

3. References to the research

1. Marsden, C.D., B.K. Mable, R. Woodroffe, G.S.A. Rasmussen, S. Cleaveland, J.W. McNutt, R. Thomas, L.J. Kennedy (2009). Highly endangered African wild dogs (*Lycaon pictus*) lack variation at the major histocompatibility complex. *J. Heredity* 100: S54–S65. doi:10.1093/jhered/esp031.
2. Marsden, C.D., R. Woodroffe, M.G.L. Mills, J.W. McNutt, S. Creel, R. Groom, M. Emmanuel, S. Cleaveland, P. Kat, G.S.A. Rasmussen, J. Ginsberg, R. Lines, J.-M. André, C. Begg, R.K. Wayne, R.K., and B.K. Mable (2012). Spatial and temporal patterns of neutral and adaptive genetic variation in the endangered African wild dog (*Lycaon pictus*). *Mol. Ecol*, 21:1379–1393. doi:10.1111/j.1365-294X.2012.05477.x.
3. Marsden, C.D., H. Verboekmoes, R. Thomas, R.K. Wayne and B.K. Mable. (2013). Pedigrees, MHC and microsatellites: an integrated approach to assessing genetic diversity in captive populations of African wild dogs. *Conserv. Genet*. 14: 171–183. doi:10.1007/s10592-012-0440-0.

4. Details of the impact

African Wild Dogs represent a unique lineage of wolf-like canids that have been classed as endangered on the IUCN's Red List of Threatened Species for the past 22 years. Currently, the worldwide captive AWD populations are managed separately in continent-specific programmes (USA, Europe, Australia and South Africa). In Europe, the captive population of AWDs is managed within a European Endangered Species Programme (EEP), an intensive form of captive management. European zoos hold half of the world's captive population of AWDs (288 individuals).[a] The EEP is overseen by a coordinator (currently based at GAIAzoo, Kerkrade, The Netherlands) and a committee; the coordinator is responsible for collecting and maintaining information on each individual in captivity, and planning for the future management of the species. However, up until 2008 this had been difficult owing to incomplete studbook records, which contain information about the pedigree and history of individuals. In addition, the studbook records were not based on genetic diversity, which is of key importance in breeding programmes.

> "*From working with this studbook since 1992 it was very clear to me that not all data were very reliable, especially the earlier ones… Before [2008] I made recommendations on best information I could get from studbook data analysis and my personal knowledge of the zoos, their breeding results the family lines... After the 2008 research we had proof of the DNA of 88% of the African wild dogs in our program*." – African Wild Dog EEP coordinator[a]

Implementation of the genetically informed management plan

The genetically informed management plan developed by researchers at the University of Glasgow was initially discussed and ratified at a meeting of the European Association of Zoos and Aquaria (EAZA) studbook management committee in Kerkrade, The Netherlands in 2008. The EAZA has statutory oversight over all member zoos involved in conservation breeding, and consists of the studbook coordinators, a vet and other zoo managers who make breeding recommendation decisions annually and meet every 3 or 4 years.

Since 2009, recommendations based on the genetic profiling of wild and captive populations of AWDs conducted by the University of Glasgow have changed the way in which the captive AWD populations across the European zoo network are managed.[a] The recommendations direct which individuals should be selected for breeding and which should be relocated and to where. Currently, 53 zoos in 16 European countries (and one in Tel Aviv, Israel), are involved.[a]

> "*Without genetic management a significant amount of diversity will be lost in the next twenty years. Based on these results, recommendations were made in August 2008 for a large number of transfers in order to compile better genetic breeding groups.*" – EAZA Annual Report (2007/08)" [b] – referring to pre-publication results of the University of Glasgow research[3]

Application of the genetically informed management plan

Dr Clare Marsden (University of California, Davis, 2010–present), who was a research student working under Mable (2006–2010), has provided an ongoing voluntary role as consulting geneticist to the studbook coordinator (2008–present). This involves a yearly consultation involving a case-by-case assessment of AWDs in each zoo. Since 2009, 98 individuals have been exchanged (always as small groups).[a] Based on the Glasgow data, animals from under-represented (and not inbred) founder lineages are identified, as are animals that need to be moved to prevent inbreeding. From this list, the coordinator and geneticist (Marsden) identify groups of males and females that make good breeding combinations from a genetic perspective, but also meet certain guidelines that increase the likelihood that the breeding group will form and successfully produce offspring.

> "*Clare [Marsden] figured out exactly which mixes would give best results, I checked if it would be possible in practical ways to bring these animals together*." – African Wild Dog EEP coordinator[a]

For example, in the 2008/9 consultation, four males in Ebeltoft zoo (Denmark) were assigned as high priorities in terms of genetic management criteria, so two high priority females were

transferred from Port Lympne Park (Kent, UK) to Ebeltoft zoo to breed with two of the males. Meanwhile, the two other Ebeltoft zoo males were transferred out to another zoo so that two females could be bonded with them.[a] Creating small breeding groups such as this reduces the chance that the males would kill the females (if two females had been introduced to four males); success is higher if introduction of male and female groups are similar in age and size (e.g. three males to three females), and where the animals were with their parents long enough to help raise a litter in a subsequent year (i.e. so they learn how to rear young).

In 2012, a breeding group needed females to bond with males in both Dortmund (Germany) and Rome (Italy) zoos. Females were available from Duisburg (Germany) and Sigean (France) zoos. The original proposal had been to combine males from Wroclaw zoo (Poland) with the Duisburg females, and Rome males with Sigean females, but this was a poor combination genetically. So instead, the genetically informed management plan enabled the selection of a better genetic match by sending Sigean females to Dortmund, and Duisburg females to Rome.[a]

Balancing genetic consultation with behavioural constraints

The genetically informed management plan also takes account of key logistical issues raised by the age, sex, and sex ratio of the group to be introduced, and how those individuals were reared. It remains difficult to implement an optimal genetically informed management plan because the best genetic matches aren't always possible. For example, some individuals who would be well matched are too old to breed, or were hand-reared and thus unable to rear offspring (typically they kill them); or the males and females are very different ages, where experience shows there is a low chance of the group breeding successfully. Often one sex is less available, making finding matches for genetic priorities difficult. Nevertheless, AWDs unsuitable for breeding, genetically speaking, may be good helpers for rearing pups or useful as companion animals – which can benefit breeding AWDs of high priority.

The genetically informed management plan also balances the desire of the zoo with what is best for the breeding programme. For instance, most zoos want to establish a breeding group, but if all zoos had breeding groups then there wouldn't be enough space for all the pups. In addition, a new zoo that hasn't bred AWDs before may have lower likelihood of successfully producing a litter. For this reason, consultations try to give the most important, high priority breeding animals to the most experienced zoos.[a] The University of Glasgow research has meant that:

> *"Zoos across Europe had to revise their studbooks, and radically re-think the husbandry, management and breeding programmes of captive wild dogs….The best scientific research makes a difference to our knowledge of a species, and how we manage it. This piece of work has done both*." – Royal Zoological Society of Scotland, Edinburgh, Annual Review 2008[c]

The management plan established by the University of Glasgow research has also initiated the early stages of genetically informed management to the USA and Australia captive breeding programmes. In 2011, Pittsburgh zoo (USA) hosted a workshop involving the African wild dog breeding coordinators of the American Zoo Association and the Australian Zoo and Aquarium Association, with Marsden (by this time Postdoctoral Research Associate at UC Davis, California) invited to share insights into the development and implementation of the Glasgow-EAZA plan.[d] It was agreed that all regions would perform similar DNA surveys in their AWD populations, with the aim of initiating a genetically informed management plan for the US and Australian captive populations. These surveys are currently underway in the USA, with 56 samples from 23 zoos currently documented.[e]

5. Sources to corroborate the impact

a. Statement from European African Wild Dog EEP coordinator (available on request)
b. European Association of Zoos and Aquaria Yearbook 2007/2008 (p10)
c. Royal Zoological Society of Scotland Annual Review 2008 (p28)
d. African Painted Dog Workshop, April 14–15, 2011 (materials also available on request)
e. Statement on USA Survey from Assistant Professor of Biology, Penn State Beaver, Monaca, USA (available on request).