

The genetics of reintroduced addax populations in Tunisia: moving towards a global management plan.

Year 1 progress report, January 2018

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Front cover photo: Addax in Jbil National Park, Tunisia. Photo by Marie Petretto, Marwell Wildlife

Back cover photo: Jbil National Park, Tunisia. Photo by Tim Woodfine, Marwell Wildlife

Acknowledgements

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We would also like to thank all of the EAZA and AZA institutions that have supported our project by providing samples to the biobanks in San Diego Zoo Global and the Royal Zoological Society of Scotland. The project would not be possible without your assistance.



1. Introduction

The Addax (*Addax nasomaculatus*) is a Critically Endangered (IUCN SSC Antelope Specialist Group, 2016) desert antelope that formerly inhabited the dunes and gravel plains of the Sahara. Once widespread and abundant in the region, addax have suffered catastrophic declines due to unsustainable hunting, habitat degradation and competition with domestic livestock, disturbance and persecution through oil exploration in prime addax habitat and political and civil unrest. As a result, indications suggest that the addax remains on the brink of extinction in the wild (Rabeil, 2016), and is locally extinct in Tunisia.

Addax have been conserved in zoological institutions in Europe, North America, Arabia and Asia and there are established and managed populations (EEP, SSP) within EAZA and AZA zoos. The EAZA addax population has been the primary source of animals for reintroduction projects in Morocco and Tunisia (Krause, 2015).

A series of historical conservation translocations has re-established three populations of addax in Tunisian national parks. Given the critical situation of addax in the wild, a meta-population model may be the only option that many former range states can adopt if they want to see the conservation and the return of the addax. While offering a pragmatic solution, the creation of meta-populations in relatively small, fenced protected areas presents many challenges. Theoretically, these small isolated groups created from few and possibly closely related founders will suffer from the effects of inbreeding and loss of genetic diversity. Mitochondrial DNA investigation¹ of fecal pellets collected in surveys of the last remaining population of wild addax in the Termit Tin Toumma region of Niger (Sahara Conservation fund 2011-16) indicates that even in this rapidly disappearing wild population, genetic diversity appears to be higher than that of the captive population from which the Tunisian population was founded. This only highlights further the need for extremely careful management to maximise the remaining genetic diversity within the Tunisian, European, North American and Arabian populations. As meta-population management becomes increasingly necessary for other populations, or even other species, the addax scenario in Tunisia also offers an unprecedented opportunity to test a number of hypotheses under real world conditions.

A collaborative project has been initiated between Marwell Wildlife, Al Ain Zoo, the Royal Zoological Society of Scotland, San Diego Zoo Global and the Tunisian Direction Générale des Forêts to evaluate the genetic diversity of addax across global ex-situ populations and the Tunisian metapopulation. The project will utilise tissue samples from addax in Tunisia, and blood as a part of veterinary screening in the ex-situ populations. Using these samples, thousands of SNP genetic markers will be identified and genotyped. The results will help inform practical decisions about the management of protected areas and the Tunisian addax population, whilst having wider implications for conservation biology.

This report summaries the progress on the project up to the end of year 1 (2017).

2. The historical and current meta-population of addax in Tunisia

An initial reintroduction of eight (4.4) EEP addax to Bou Hedma National Park (BHNP), Tunisia was carried out in September 1985 with an additional six females reintroduced from the USA in March 1988 (Figure 1). In 1993, six (3.3) addax were translocated from BHNP to the neighbouring annex national park of Haddej (HNP). In February 2007, 15 (5.10) addax were translocated from Bou Hedma to Jbil (JNP), with a further four (1.3) translocated from Bou Hedma to Senghar-Jabbes (SJ) to establish new populations in these two parks. The population in JNP was augmented in December 2007 when 13 (4.9) addax were transferred

¹ RZSS WildGenes, based on 18 wild samples from Termit and 76 captive samples from across the Europe and Middle East, ongoing study.

from the EEP and North American Species Survival Plan (SSP) with the objective of creating a more genetically diverse population. The remaining addax herd in BHNP (2.2 individuals) was transferred in 2016 to HNP.

The December 2017 census of addax in each National Park estimated populations of 59 in Haddej, 16 in Jbil and 33 in Senghar-Jabbes, with a total meta-population of addax in Tunisia of approximately 110 addax.

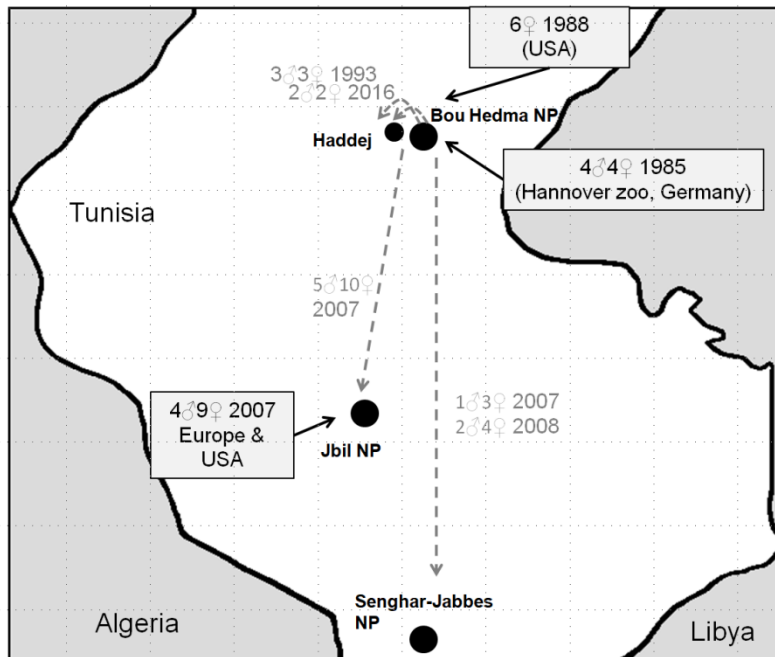


Figure 1. The three Tunisian national parks that host addax populations along with details on their founders

3. Collection of tissue samples from addax populations in Tunisia

The collection of tissue samples from the Tunisian addax population began in early August 2017 when the Tunisian field biology team, consisting of Marie Petretto, Chawki Najjar, Abdelkader Chetoui and a number of Tunisian students, visited Jbil National Park for the first biopsy darting mission (Figures 2 & 3); during the following months all the three protected areas with addax were intensively surveyed and individuals identified. The students from the Faculty of Science at the University of Tunis and the National Veterinary School of Sidi Thabet were included to provide some field support for the collection and processing of samples, and to provide them with an opportunity to gain experience in wildlife conservation in a field environment. Veterinary health assessments of the addax were conducted at the same time.

The sampling operation collected biopsy tissue samples from 104 of 108 addax that were directly observed in the Tunisian parks.

Additional historic and current population records were collated along with information on historic management interventions to compile a complete picture of historical management of addax populations in each park.



Figure 2. Marwell’s Tunisian team (Marie Petretto, Chawki Najjar and Abdelkader Chetoui) together with the volunteer students in Master of Populations Ecology (left) and Veterinary Medicine (right)



Figure 3. Tunisian veterinary students assisting with field work. Left, Youssef taking identification pictures during darting; Right, Nadia and Habib helping Marie Petretto to retrieve the skin samples from the biopsy needles and reload new darts

3.1 Biopsy darting procedures

The individuals were darted using a Dan-inject® rifle with dart syringes and large-gauge needles. The needles retained a small skin plug when they impacted and detached from the addax (Figures 4, 5 & 6). Individual identification was required to avoid duplicate sampling. Tissue samples (104 skin plugs) were collected from all adult, sub-adult and juvenile (under 1-year old) addax in the three parks, with exception of one female and three young calves in HNP due to ethical and welfare considerations in biopsy darting very young animals.



Figure 1. Marwell’s team approaching a group of addax to collect biopsy samples in Jbil NP



Figure 2. A male addax showing no apparent adverse reaction to the dart impact



Figure 3. Marie collecting the dart with a tissue sample after it rebounded from the Addax's leg

3.2 Missions

The first mission to Jbil National Park took place on the 4th – 6th August 2017 where 81% of all observed addax were sampled. A second mission took place on the 12th – 14th of December 2017 to complete sample collection; intensive searching was conducted to ensure all individuals were sampled. Additional missions took place to Senghar-Jabbes NP and Haddej NP in October and November 2017, respectively, to collect samples from nearly all observed addax within the parks (Table 1).

Table 1. The number of addax sampled in each age category in each park

Site	Dates (2017)	Adult		Sub-adult		Juvenile		Calf	Total	% Sampled
		M	F	M	F	M	F			
Jbil mission 1	4-6 August	6	5	0	0	0	2	0	13	81%
Jbil mission 2	12-14 December	1	2	0	0	0	0	0	3	19%
Senghar-Jabbes	2-3 October	15	12	1	0	1	4	0	33	100%
Haddej	9-13 November	19	25 ^a	3	2	5	1	0 ^b	55	93%

^a One female initially retained the dart and was not re-sampled

^b Three young calves weren't sampled due to ethical and welfare considerations

In total, 104 biopsy samples were collected from addax in the Tunisian parks, with an additional four samples collected during veterinary procedures or post-mortem from Jbil NP. The samples were couriered to the WildGenes Laboratory at the Royal Zoological Society of Scotland in January 2018 ahead of laboratory analyses.

4. Samples from the EEP addax population

A number of requests for tissue and blood samples have been sent to EEP institutions over the course of 2017. However, samples can only be collected when an animal is restrained for health or veterinary reasons resulting in slow sample collection. To date, the WildGenes Laboratory have a total of 98 samples (table 2), however, the hair samples may yield little DNA.

Table 2. Samples received by RZSS to date

Location	Date	Number of samples	Sample type
Al Ain Zoo	2017	29	Blood
Kolmarden	October 2017	26	Blood
Bratislava	Archived	5	Hair
Safari de Peaugres	Archived	5	Tissue & hair
Zlin	Archived	1	Hair
Al Ain Zoo	Archived	32	Blood

5. Samples from the SSP addax population

Addax samples have been intermittently and opportunistically banked in San Diego Zoo Global's Frozen Zoo® since the mid-1970s. In addition, the SSP was recently intensively sampled as part of that program's efforts to resolve the population's poorly known pedigree. To date, the combined total number of animals for which samples are available is 301 SSP addax. Unfortunately, samples for 80 of the 301 animals are blood spots on FTA (filter paper) cards that may ultimately prove insufficient for the proposed molecular analyses. The remaining 221 samples are all high quality tissue and/or blood samples. To date, SNP data have already been collected for 125 individuals; 15 individuals were genotyped using the Illumina BovineHD BeadChip, 100 individuals were genotyped through restriction site-associated DNA (RAD) sequencing, and 10 individuals were genotyped using both methodologies. San Diego Zoo Global will be coordinating the SNP genotyping (RAD sequencing) of the remainder of the currently available individuals throughout 2018.

6. Communication

We began to disseminate information on the project by publishing a short Conservation News article in the IUCN SSC Antelope Specialist Group Gnuletter: Riordan, P., Gilbert, T.C., Petretto, M., Craig, M.S., Banfield, L.M., Ivy, J., Senn, H., Abib, H. & Nouioui, M. (2017): The genetic diversity of addax *Addax nasomaculatus* in reintroduced and ex-situ managed populations. Gnuletter, 34(1), p21 (Appendix B).

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Appendix A: List of biopsy sampled collected from the Tunisia addax population

Animal ID	sex	Age	Park	Date	number of samples per individual
HNP 01	M	Adult	Haddej	09/11/17	2
HNP 02	F	Adult	Haddej	11/11/17	1
HNP 03	M	Adult	Haddej	09/11/17	1
HNP 04	F	Adult	Haddej	09/11/17	1
HNP 05	M	Adult	Haddej	11/11/17	1
HNP 06	F	Adult	Haddej	12/11/17	1
HNP 07	M	Adult	Haddej	09/11/17	1
HNP 08	F	Adult	Haddej	11/11/17	1
HNP 10	F	Adult	Haddej	10/11/17	2
HNP 11	M	Adult	Haddej	09/11/17	2
HNP 12	F	Adult	Haddej	13/11/17	1
HNP 13	F	Adult	Haddej	12/11/17	1
HNP 14	M	Adult	Haddej	09/11/17	2
HNP 15	M	Adult	Haddej	09/11/17	2
HNP 16	M	Adult	Haddej	11/11/17	0.5
HNP 17	M	Adult	Haddej	09/11/17	1
HNP 18	M	Adult	Haddej	09/11/17	1
HNP 19	M	Adult	Haddej	09/11/17	1
HNP 20	M	Adult	Haddej	11/11/17	1.5
HNP 21	F	Adult	Haddej	11/11/17	1
HNP 22	M	Adult	Haddej	10/11/17	1
HNP 23	M	Adult	Haddej	12/11/17	1
HNP 24	F	Adult	Haddej	10/11/17	1
HNP 25	F	Adult	Haddej	12/11/17	0.5
HNP 26	F	Adult	Haddej	10/11/17	1
HNP 27	F	Adult	Haddej	10/11/17	1
HNP 28	F	Adult	Haddej	10/11/17	3
HNP 30	F	Adult	Haddej	12/11/17	0.5
HNP 31	F	Adult	Haddej	10/11/17	1
HNP 32	F	Adult	Haddej	10/11/17	1
HNP 33	M	Adult	Haddej	10/11/17	1
HNP 34	F	Adult	Haddej	10/11/17	1
HNP 35	F	Adult	Haddej	10/11/17	2.5
HNP 36	F	Adult	Haddej	10/11/17	1
HNP 37	M	Adult	Haddej	10/11/17	2
HNP 38	F	Adult	Haddej	10/11/17	0.5
HNP 39	M	Adult	Haddej	10/11/17	0.5
HNP 40	F	Adult	Haddej	13/11/17	1
HNP 41	F	Adult	Haddej	10/11/17	1
HNP 42	F	Adult	Haddej	10/11/17	1
HNP 43	F	Adult	Haddej	11/11/17	1
HNP 44	M	Adult	Haddej	12/11/17	1
HNP 45	M	Adult	Haddej	13/11/17	1
HNP 46	F	Adult	Haddej	12/11/17	1
HNP 48	M	SubAdult	Haddej	13/11/17	1
HNP 49	M	SubAdult	Haddej	10/11/17	0.5
HNP 50	M	SubAdult	Haddej	10/11/17	1
HNP 51	F	SubAdult	Haddej	13/11/17	1
HNP 52	F	SubAdult	Haddej	13/11/17	1.5
HNP 53	M	Juvenile	Haddej	13/11/17	1
HNP 54	M	Juvenile	Haddej	12/11/17	1
HNP 55	M	Juvenile	Haddej	12/11/17	1
HNP 56	M	Juvenile	Haddej	12/11/17	1
HNP 57	M	Juvenile	Haddej	13/11/17	1
HNP 58	F	Juvenile	Haddej	12/11/17	2
JNP 01	F	Juvenile	Jbil	05/08/17	1

Animal ID	sex	Age	Park	Date	number of samples per individual
JNP 02	M	Adult	Jbil	13/12/17	2.5
JNP 03	M	Adult	Jbil	05/08/17	1
JNP 04	F	Adult	Jbil	05/08/17	1
JNP 05	F	Adult	Jbil	05/08/17	1
JNP 06	F	Adult	Jbil	05/08/17	1
JNP 07	M	Adult	Jbil	05/08/17	1
JNP 08	M	Adult	Jbil	13/12/17	1
JNP 09	M	Adult	Jbil	05/08/17	1
JNP 10	M	Adult	Jbil	05/08/17	1
JNP 11	F	Juvenile	Jbil	05/08/17	1
JNP 12	F	Adult	Jbil	05/08/17	1
JNP 13	F	Adult	Jbil	05/08/17	2
JNP 14	M	Adult	Jbil	06/08/17	1
JNP 15	F	Adult	Jbil	13/12/17	1
JNP 16	F	Adult	Jbil	13/12/17	1
JNP A*	M	Adult	Jbil	12/02/13	1
JNP B*	M	Adult	Jbil	23/01/14	1
JNP C*	M	Adult	Jbil	25/02/13	1
JNP D*	F	Calf	Jbil	12/12/12	1
SJ01	M	Adult	Senghar-Jabbes	02/10/17	1
SJ02	M	Adult	Senghar-Jabbes	02/10/17	1
SJ03	M	Adult	Senghar-Jabbes	02/10/17	1
SJ04	M	Adult	Senghar-Jabbes	02/10/17	1
SJ05	M	Adult	Senghar-Jabbes	02/10/17	1
SJ06	M	Adult	Senghar-Jabbes	02/10/17	1
SJ07	M	Adult	Senghar-Jabbes	02/10/17	1
SJ08	M	Adult	Senghar-Jabbes	02/10/17	1
SJ09	M	Adult	Senghar-Jabbes	02/10/17	3
SJ10	M	Adult	Senghar-Jabbes	02/10/17	1
SJ11	M	Adult	Senghar-Jabbes	02/10/17	1
SJ12	F	Adult	Senghar-Jabbes	02/10/17	1
SJ13	M	Adult	Senghar-Jabbes	03/10/17	1
SJ14	M	Juvenile	Senghar-Jabbes	03/10/17	1
SJ15	F	Juvenile	Senghar-Jabbes	03/10/17	1
SJ16	F	Juvenile	Senghar-Jabbes	03/10/17	0.5
SJ17	F	Juvenile	Senghar-Jabbes	03/10/17	1.5
SJ18	M	SubAdult	Senghar-Jabbes	03/10/17	0.5
SJ19	F	Adult	Senghar-Jabbes	03/10/17	1
SJ20	F	Juvenile	Senghar-Jabbes	03/10/17	1
SJ21	F	Adult	Senghar-Jabbes	03/10/17	1
SJ22	F	Adult	Senghar-Jabbes	03/10/17	1
SJ23	M	Adult	Senghar-Jabbes	03/10/17	1
SJ24	F	Adult	Senghar-Jabbes	03/10/17	2
SJ25	F	Adult	Senghar-Jabbes	03/10/17	1
SJ26	F	Adult	Senghar-Jabbes	03/10/17	1
SJ27	M	Adult	Senghar-Jabbes	03/10/17	1
SJ28	F	Adult	Senghar-Jabbes	03/10/17	1
SJ29	F	Adult	Senghar-Jabbes	03/10/17	0.5
SJ30	F	Adult	Senghar-Jabbes	03/10/17	1
SJ31	F	Adult	Senghar-Jabbes	03/10/17	1.5
SJ32	M	Adult	Senghar-Jabbes	03/10/17	1
SJ33	F	Adult	Senghar-Jabbes	03/10/17	1

*samples collected through post-mortem sampling and veterinary health checks

Appendix B : Gnusletter article

The genetic diversity of addax *Addax nasomaculatus* in reintroduced and ex-situ managed populations

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Marwell Wildlife, Al Ain Zoo, the Royal Zoological Society of Scotland and San Diego Zoo Global have initiated a project with the Tunisian Direction Générale des Forêts to evaluate the genetic diversity of addax across global ex-situ populations and the Tunisian metapopulation. The project will collect tissue samples from addax in Tunisia through biopsy darting, and blood as a part of veterinary screening in the ex-situ populations. Using these samples, thousands of SNP genetic markers will be identified and genotyped to develop better metapopulation management policies and ensure these remaining addax are able to persist and breed into the future. Addax were once widespread and abundant across the dunes and gravel plains of the Sahara, but are now on the brink of extinction in the wild. The species has been conserved and bred in zoological institutions in Europe, North America, the Arabian Peninsula and Asia, and was released into fenced protected areas in Morocco and Tunisia between 1985 and 2007. The Tunisian metapopulation is split between three National Parks and this project will guide efforts to ensure these animals are managed effectively as a national herd.

Given the critical situation of addax in the wild, a metapopulation approach may be the only option that many former range states can adopt if they want to see the conservation and the return of the addax. Metapopulations consist of clusters of often small subpopulations that are interlinked to varying degrees. While offering a pragmatic solution, the creation of metapopulations in relatively small, fenced protected areas presents challenges. Theoretically, these small isolated groups created from relatively few and possibly closely related founders may suffer inbreeding depression and loss of genetic diversity. The metapopulation is likely to need management to offset potential problems and maximise the genetic diversity across the Tunisian, European, Arabian and North American populations. As metapopulation management becomes increasingly necessary for other populations and species, the addax scenario in Tunisia also offers an unprecedented opportunity to test a number of hypotheses under real world conditions. We anticipate the results having wider implications for conservation biology, but will also help inform practical decisions about the management of protected areas and the Tunisian addax population.

This project will begin in 2017 and run until 2019. If you would like any further information, please contact Dr Philip Riordan, Head of Conservation Biology at Marwell Wildlife:
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