

Prioritizing the collection of samples for genetic rescue

Participants

Jon Ballou, Taylor Callicrate, Dalia Conde (convenor), Danny de Man, Kyle Flesness, Nate Flesness, Myfanwy Griffith, Jim Guenter, Markus Gusset, Heribert Hofer, Jamie Ivy, Mansoor Al Jahdhami, Melissa Kenney (facilitator), Nian-Hong Jang-Liaw, Sarah Long, Paul Pearce-Kelly, Andrea Putnam, Roopali Raghavan, Jorge Rodríguez, Oliver Ryder (convenor), Anke Schirmer, Karin Schwartz, Chih-Chin Shih, Lee Simmons, Boripat Siriaroonrat, Johanna Staerk (convenor), Sara Sullivan, Kazu Takami, Kathy Traylor-Holzer, Eric Tsao

Aim

The aim was to brainstorm ideas on the decision opportunity of prioritizing actions for the collection and storage of live cells for genetic rescue and was a follow-up on the CBSG workshop "Genetic Rescue" at the CBSG Annual Meeting 2015. The group identified a wide range of factors to consider in making prioritization decisions and brainstormed lots of options for the collection, storage, and prioritization for a wide range of species. These ideas will provide the basis of a decision framework that will be developed to decide on actions that can further advance genetic rescue as a tool in the conservation toolkit.

Background

Genetic Rescue is the response to an extinction crisis. In many threatened species, genetic diversity is low due to small or fragmented populations with little genetic exchange. Inbreeding can lead to the loss of favorable traits and weakens the population's ability to adapt to changing environments, such as during climate change. As defined in the CBSG Annual Meeting 2015, genetic rescue is an increase in population-level viability through the re-introduction of previously lost genetic material by cell-based human intervention. It involves utilizing preserved and banked tissue samples, both reproductive and somatic across a variety of technological means to add genetic diversity and/or produce viable offspring for critically endangered species. It has the greatest potential for impact where traditional means of species recovery by live animal transfer are not practical or possible. Technologies include artificial insemination and *in vitro* fertilization along with induced stem cell development and applications of cloning technology. Numerous challenge is determining the method to prioritize the species in need of rescue and another is the lack of availability of suitable samples. In this workshop we explored ideas on objectives, options and alternatives for prioritizing actions for the collection and storage of live cells for genetic rescue.

Process

In the working group session we focused on the following four topics:

1) Factors to consider in making prioritization decisions

To make decisions one has to allocate limited resources and therefore prioritize. Here, the group discussed how and what to prioritize.

2) Datasets

Good decision-making requires quality data. Here, the group discussed which datasets could possibly be useful.

- 3) Options for the preservation and collection of genetic material One challenge is the lack of suitable samples for genetic rescue. Here, the group discussed how to advance the preservation and collection of genetic samples.
- Discussion of reservations and concerns
 Considering values and concerns and including stakeholders are an important part of each decision-making process. Here the group discussed possible reservations and concerns.

1) Factors to consider in making prioritization decisions

In the first session, the group identified a wide range of factors to consider in making prioritization decisions for genetic rescue, given limited resources (time, money). Major factors included species prioritization based on conservation status and taxonomic uniqueness, the species role in the ecosystem, the accessibility of samples (e.g. logistics, permits, opportunity) and the use of existing *exsitu* management for species conservation as an opportunity to collect samples. Other factors included costs, facility options (e.g. safety, network, capacity), public values, animal welfare, no alternative methods, existing conservation plans, sampling in emergency situations, etc. For the full list of factors, see below (List 1).

2) Datasets

In order to make good decisions quality data are important. The group therefore brainstormed datasets that could be useful in developing a decision framework for the prioritization of actions for genetic rescue, ranging from datasets of prioritizations to databases on e.g. banked tissue and samples. For the full list of datasets, see Table 1.

3) Options for the collection and preservation of genetic material

The group brainstormed lots of options for the collection, storage, and prioritization for a wide range of species. Some of these included opportunistic sampling in captivity, capacity building in local regions or amongst zoo veterinarians and making banking a compulsory part of the accreditation process. Further ideas included global and local coordination among biobanks, a centralized database, simplifying bureaucracy by e.g. standardized protocols, MOUs, or a Nagoya exemption for zoos. For an extended list of options, see below (List 2).

In a second session the group thought more deeply about options divided into three topics: options in 1) single species sample collection, 2) collection from zoos and 3) collection in the wild and how to present these options to a financial donor. The focus was on how to prioritize sampling and what funding is needed for further research to make that sampling possible, more efficient and/or more effective.

Single species sample collection: Species should be prioritized based on threat status, isolation, and number of populations as well as their taxonomic uniqueness. Sampling of species that already have cell lines should be considered a priority in order to add more diversity to those. More research is needed to develop methods for incorporating genetic samples into live populations and to identify baseline genetic variation and population dynamics of endangered species. Funding should be based on flagship species that can serve to establish necessary infrastructure also for other species. Using corporate connections or companies mitigation procedures are some possibilities to acquire further funding for biobanks.

Collection in zoos: Species should be prioritized based on status (e.g. threat status, uniqueness etc.). Further, raising awareness of the topic and integrating it into existing protocols (e.g. during husbandry, veterinarian work, etc.) should be a priority. There is a need for further research into genetics and reproductive systems of many species, as well as better methods and training. Another priority is to

establish a global network of biobanks. This could be achieved with the help of the zoo network and possibly human biobanks in order to simplify logistic issues. Current barriers that need to be overcome are legislative barriers, access to technology for sampling and storage as well as security and maintenance issues in countries where this is challenging. Making use of zoos provides the advantage that animals are accessible, the staff is already engaged, and a communication structure is already in place.

For funding the type of donor is important. A challenge herewith could be to communicate a pioneering approach vs. a proven approach and focus should be on successful examples. Genetic rescue needs to be communicated as avoiding "having many eggs in one basket" rather than as a last resort. Collecting from species that are at brink of extinction is urgent and needs to be done as long as they still exist. Funding at the systematic level might be easier to obtain than at just at the species level.

Collection in wild: The wild working group identified the IUCN habitat/region assessments as an important component for prioritization and suggested to fill up sampling gaps by assessing existing samples to identify their region of origin. An idea was to use existing field researchers for sampling or plan new expeditions. There should be investments into the development of sampling and sample processing protocols and there should be more funding for sample processing centers in various regions and for long-term stable storage. The group also expressed the need for more research concerning sampling methods for invertebrates (e.g. pollinators).

4) Discussion of reservations and concerns

The group discussed reservations and concerns towards genetic banking and rescue. Concerns were: the use of samples mainly for research instead of conservation, a delay of measures and actions until the point at which the population is too small already, miscommunication of the topic to the public (Jurassic park scenarios/ cloning vs. preserving genetic diversity) and that there might be problems with religious beliefs, even though none were identified. There was further discussion of the importance of sampling now while there is still genetic diversity left, which would also pose less sampling risk to the population. There was concern that if genetic rescue is perceived as the last measure, there might be reluctance due to the belief that hope is already lost. Therefore, one needs to frame this process as part of a conservation success story or successful plan.

There is a need for more global coordination, to increase awareness of the topic but also in directing sampling efforts. Genetic rescue should become an on-going discussion in CBSG and the group would like to bring WAZA and the IUCN into the discussion.

Recommended actions:

- Recommendation of the topic to the Committee for Population Management and the Conservation Committee of WAZA for discussion until the 2017 CBSG meeting (Danny de Man & Nate Flesness)
- Formulation of a policy or extension of a policy about the how to actualize the collection of cellular material for genetic rescue that can be part of the mission of the constituency. Regional association representatives can help provide enforcement that this will actually happen in the regions or be endorsed in the regions (Oliver Ryder & small team)

- A regular newsletter to keep communication going within this group and the larger CBSG group about progress, success stories, goals, methodologies (Johanna Staerk & group, ca. 4x per year, starting November 2016)
- Presentation to WAZA at the 2017 meeting in Barcelona with summary of the 2015 and 2016 CBSG Genetic Rescue Working Group reports and the progress to date

List 1 – Factors to consider for prioritization decisions:

- Species prioritization
 - Status (IUCN / CITES / EDGE/ AZE)
 - Population size
 - Conservation reliance
 - Surrogates available
- Accessibility of samples feasibility for collection
 - Population size
 - o Knowledge
 - o Legislation
 - o Wild
 - o Captive
 - o **Ownership**
 - Logistics (time, staff)
 - o Physical accessibility (transport, reachability, distance)
 - o Safety
 - Willingness to share
 - o Confiscation
- Collection risk for population or species
 - o Risk of collection (immobilization)
 - Risk of disease transfer
 - Risk to perception of the program
- Costs
 - Sustainable funding
 - Costs of collection and storage resources for space, staff, time,
- Facility options
 - Longitudinal safety of storage reliability and commitment
 - o Capacity
 - o Availability of resources
 - o Duplication strategic redundancy
 - Partner networks (no duplicate efforts)
 - Taxonomic diversity of the bank
 - o Safety
- Values of public (Cultural / Economic / Ecological)
- Animal welfare
- Science issues (Research potential)
- Data management plan
- Crisis / Emergencies

- No alternative methods exist
- Conservation plan for use of stored samples
- Conservation roles augmentation, assurance, research

List 2 - Options:

- Use existing moments of sampling opportunity + training and support of staff (zoos, confiscations, Taxon Advisory Group teams, field-workers, museums, private collections, Fish and Wildlife Service (e.g. government authorities), rescue & rehabilitation centers, road-kill, beachings, hunting, placenta, pharma, scientists, rhino horn, ear notching etc., consult with human banks)
- Proactive sampling, training and support
- Global or local coordination linking of existing biobanks and expansion of network, with efficient transportation network, central or connected databases (Zoological Information Management System?), multiple location of redundant samples
- Ease bureaucracy (protocols, language etc., Nagoya exemption for zoos, MOU of collections)
- Make banking part of zoo accreditation
- Add to IUCN guidelines, rapid response team
- Use/ encourage new technology (drones, human bankers, price for new innovation, environmental DNA...)
- Provide incentives for establishing banks/ contribution
- WAZA resolution on banking

Table 1 Databases/ Sources of Information:

Name	Description	Contact / Link
NCBI – GenBank	Sequence database	https://www.ncbi.nlm.ni
		h.gov/genbank/
Barcode of Life (BOLD,	DNA barcoding, mtDNA sequence	http://www.barcodeoflif
iBOL, CBOL)	databases	e.org
ZIMS	Zoological Information Management	www.species360.org
	System	
CITES	Convention on International Trade in	https://www.cites.org
	Endangered Species of Wild Fauna and	
	Flora	
EDGE of Existence	Evolutionarily Distinct & Globally	http://www.edgeofexiste
	Endangered; species prioritization	nce.org
PANTHER	(Protein ANalysis THrough Evolutionary	http://www.pantherdb.o
	Relationships) Classification System	rg
WoRMS	World Register of Marine Species;	http://www.marinespeci
	taxonomic register	es.org
Dryad	Digital repository for data underlying	https://datadryad.org
	scientific and medical publications	
GBIF	Global Biodiversity Information Facility	http://www.gbif.org
UNEP WCMC	UNEP World Conservation Monitoring	https://www.unep-
	Centre	wcmc.org
ConGRESS	Conservation Genetic Resources for	http://www.congressgen
	Effective Species Survival	etics.eu , Andrew Pletnum

GGBN	Global Genome Biodiversity Network	http://www.ggbn.org/gg bn_portal/
NZG	National Zoological Garden of South Africa	Antoinette Koetze
GGI	The Global Genome Initiative (GGI),	https://ggi.si.edu (Pierre
	Smithsonian	Comizzoli)
IZW	Leibniz Institute for Zoo and Wildlife	http://www.izw-
	Research (frozen samples)	berlin.de/welcome.html
Millennium Seed Bank	Royal Botanic Gardens, Kew (plants)	http://www.kew.org/scie
		nce-
		conservation/collections/
		millennium-seed-bank
EAZA Conservation	Conservation efforts database	Merel Zimmerman
Database		
EAZA DNA bank	Frozen tissue (?)	
Australian Frozen Zoo	Frozen live cells	http://australianfrozenzo
		o.org.au
Frozen Ark	Frozen live cells	https://frozenark.org
San Diego Frozen Zoo	Frozen live cells	http://institute.sandiegoz
		oo.org/resources/frozen-
Auduk en Netane	Free on line celle	Z00®
Audubon Nature	Frozen live cells	
Contor for the Research	Note: this center may now have a unierent	
of Endangered Species	liame.	
(CRFS)		
Henry Doorly Zoo	Bank of more than 20,000 samples of	http://www.omahazoo.c
Omaha	frozen reproductive cells from over 50	om/conservation/reprod
	species	uctive-sciences/
ATCC	American type culture collection	https://www.atcc.org/
FAO	Food and Agriculture Organization of the	http://www.fao.org/
	United Nations (wild relatives of domestic	
	species)	
DPZ	German Primate Center, Goettingen;	http://www.dpz.eu/en/h
	Frozen samples	ome.html
Kuwait National	Under development – see CBSG Update, pg.	Pierre Comizzoli
Biobank	4, October 2015	
	http://www.cbsg.org/sites/cbsg.org/files	
	/eUpdate_Files/October_2015_EN.pdf	
NIES Japan	National Institute for Environmental	http://www.nies.go.jp/bi
	Studies – Genetic Resource Banking for	ology/eng/res_E/end/ind
	Endangered Species in Japan	ex.html
AArk	Amphibian Ark	http://www.amphibianar k.org
Tree of Life Web	Taxonomy	http://www.tolweb.org/
Project		
Library of Life	California Academy of Sciences	http://www.calacademy. org/library-of-life
ZPO	Zoological Park Organization of Thailand	http://www.zoothailand.
		org/

ISBER	International Society for Biological and Environmental Repositories	http://www.isber.org/
ILRI	The International Livestock Research Institute	http://azizi.ilri.org
SpecimenCentral	Global Biobank Directory	http://specimencentral.c om/biobank-directory/
IUCN SSC <i>ex-situ</i> guidelines	IUCN SSC Guidelines on the Use of <i>Ex situ</i> Management for Species Conservation	http://www.cbsg.org/co ntent/iucn-ssc- guidelines-use-ex-situ- management-species- conservation-en-2014