GENETIC CONSERVATION

Prof. Dr. Akin Pala
Twitter: @akinpala

Conservation

- Conservation of endangered species so they won't be extinct.
  - Wildlife conservation
  - Sea turtles, Siberian tigers, bald eagle, Brown spider monkey…
- Conservation of breeds so we can keep that variation within species.
  - Over 50 horse breeds in Europe alone, many of them endangered
  - Some local cattle breeds
  - There are over 200 sheep and goat breeds, including locals.

Definitions

- A **species:** abbreviated **sp.**
  - plural form *species is* abbreviated **spp.**
- Species: The largest group of organisms where two hybrids can reproduce fertile offspring, typically using sexual reproduction.
- A **breed** is a specific group of domestic animals having homogeneous appearance (phenotype), homogeneous behavior, and/or other characteristics that distinguish it from other organisms of the same **species** and that were arrived at through selective **breeding**.
  - The term subspecies is sometimes used for wild animals.

Cats and Dogs

- Dog: Golden retriever, bulldog, German shepherd, Chihuahua, pitbull
- Cat: Siamese cat, Persian cat, Turkish Angora

International Union for the Conservation of Nature (IUCN) list

- **Extinct (EX)**
- Examples: Dinosaurs, Bali tiger, Barbary lion, Caribbean monk seal, Carolina parakeet, Caspian tiger, dodo, dusky seaside sparrow, eastern cougar, golden toad, Japanese sea lion, Javan tiger, Labrador duck, passenger pigeon, Schomburgk's deer, Steller's sea cow, western black rhinoceros

International Union for the Conservation of Nature (IUCN) list

- **Extinct in the wild (EW)**
- Captive individuals survive, but there is no free-living, natural population.
- Examples: Catarina pupfish
- Hawaiian crow
- Père David's deer
- Scimitar oryx   Antelope species
- Socorro dove
- Wyoming toad

IUCN list, Critically endangered (CR)

- Faces an extremely high risk of extinction in the immediate future.
- African wild ass
- Alabama cavefish
- Blue-throated macaw
- Arakan forest turtle
- Asiatic cheetah
- Sumatran Tiger
- Brown spider monkey

Indonesian island of Sumatra

IUCN list, Endangered (EN)

- Faces a very high risk of extinction in the near future.
- African wild dog (near critically endangered)
- Asian elephant
- Blue whale
- Snow leopard

- Tasmanian devil

IUCN list, Vulnerable (VU)

- Faces a high risk of extinction in the medium term.
- African grey parrot
- African bush elephant
- African lion
- Cheetah
- Galapagos tortoise
- Golden hamster
  - Vulnerable in the wild
  - North of Syria and the south of Turkey, in arid habitats
- Whale shark
- Hippopotamus
- Komodo dragon
- Lesser white-fronted goose
- Maned sloth
- Polar bear
- Yak
- Great white shark
- American crocodile
- Dingo
- King cobra

IUCN list, Near-threatened (NT)

- May be considered threatened in the near future.
- Asian golden cat
- Emperor Goose
- Emperor Penguin
- Leopard
- Jaguar
- White rhinoceros
- Striped hyena
- Tiger shark
- White eared pheasant

IUCN list, Least concern (LC)

- No immediate threat to species' survival.
- American alligator
- Olive baboon
- Bald eagle
- Brown bear
- Brown rat
- Cane toad
- Common wood pigeon
- Cougar
- Common frog
- Ciraffe
- Grey wolf
- House mouse
- Wolverine
- Human

Conservation Priority Livestock Breeds 2015

- Critical: Estimated global population less than 2,000.
- Threatened: Estimated global population less than 5,000.
- Watch: Estimated global population less than 10,000.
- Recovering: Breeds that were once listed in another category and have exceeded Watch category numbers but are still in need of monitoring.
- Study: Breeds that are of genetic interest but either lack definition or lack genetic or historical documentation.

Conservation Priority Livestock Breeds 2015

	Critical	Threatened	Watch	Recovering	Study
Cattle	**Canadienne** Dutch Belted **Florida Cracker** Kerry Lincoln Red **Milking Devon** Milking Shorthorn – Native[1] **Randall** or **Randall Lineback** **Texas Longhorn**[2]	Ancient White Park **Pineywoods** Red Poll	Ayrshire Galloway Guernsey	Ankole-Watusi Belted Galloway Devon or Beef Devon Dexter Highland	**Chirikof Island** **Criollo** (North Central Mexican)
Goats	Arapawa **San Clemente**		**Spanish**	**Myotonic** or **Tennessee Fainting** Oberhasli	Golden Guernsey
Pigs	**Choctaw** **Mulefoot** **Ossabaw Island**	Gloucestershire Old Spots **Guinea Hog** Large Black **Red Wattle** Tamworth	**Hereford**		Saddleback
Rabbits	**American Chinchilla**	**American** Belgian Hare Blanc de Hotot Silver **Silver Fox**	Beveren **Giant Chinchilla** Lilac Rhinelander	Crème d'Argent	Harlequin
Sheep	**Florida Cracker** **Gulf Coast** or **Gulf Coast Native** **Hog Island** Leicester Longwool **Romeldale / CVM** **Santa Cruz**	Black Welsh Mountain Clun Forest Cotswold Dorset Horn **Jacob – American** **Karakul – American** Lincoln **Navajo-Churro** **St. Croix**	Oxford Shropshire **Tunis**	**Barbados Blackbelly** Shetland Southdown Wiltshire Horn	

http://albc-usa.etapwss.com/images/uploads/docs/PriorityLivestock2015_lowres.pdf

Conservation Priority Equine Breeds 2015

	Critical	Threatened	Watch	Recovering	Study
Donkeys	Poitou	**American Mammoth Jackstock**		Miniature Donkey	
Horses	**American Cream** **Canadian** Caspian Cleveland Bay **Colonial Spanish Strains** [1] **Banker** [2,4] **Choctaw** [2,3] **Florida Cracker** [2] **Marsh Tacky** [2] **Santa Cruz** [2] **Wilbur-Cruce** [2] Dales Pony Galiceño Hackney Horse **Morgan – Traditional** [5] **Newfoundland Pony** Shire Suffolk	Akhal-Teke **Colonial Spanish – Combined** [1] Dartmoor Exmoor Lipizzan	Clydesdale Fell Pony Gotland Irish Draught **Mountain Pleasure/ Rocky Mountain**	Belgian	

http://albc-usa.etapwss.com/images/uploads/docs/PriorityLivestock2015_lowres.pdf

Why Diversity?

- It is necessary to keep diversity in livestock and in wild life.
- Decreased variety in wild life leads to extinction of species.
- Fitness is the ability to produce, as opposed to being strong.
- This can be because of inbreeding depression or lost genes that are still needed.

Methods

- Keeping live animals
  - Genetic Diversity maintenance
- Cryogenic Storage
  - Deep-freezing of sperm and oocytes
  - Deep-freezing of embryos
  - Storage of DNA

How?

- Different lines of animals can be kept to prepare for a future that requires attributes not needed today.
- The different lines can be determined using factor analysis.
- Separate lines then can be merged by random mating; making way to a herd that has the variance of the original herd.
- Alternatively, biological material can be stored cryogenically.
- Live animals can adapt to environmental changes in time and may prove more useful though keeping live animals may cost more than cryogenic storage of biological material.

Why keep old things?

- Humans like old things and try to keep old structures and memories, such as old buildings and photographs.
- People bring children into the World and want them to follow their footsteps.
- They try to reach out in time backwards by keeping old things and forward by having children.
- Humans like to see old things from their early life to forget the fact that their time is passing.

Why keep old things?

- There is an emotional drive in keeping those animals in the World.
- Wild animals are widely regarded as part of the World’s heritage and it is the duty of present generation to keep them for the future ones.
- We did not inherit those from the past generation; we borrowed them from the next generation (an old-Native-American-saying).

Why keep animals?

- The animals may have possible benefits to the industry in the future.
- Much genetic improvement in plants has been obtained by introducing genes from wild, “unproductive” species into cultivated crop plants today.
  - Benefits of crossbreeding and how progeny is sometimes superior to the parents: hybrid animals.

Examples

- A gene preventing flounder from freezing is introduced to a domestic tomato breed.
- Another example of a useful character would be the isolated population of feral pigs found on Ossabaw Island, a coastal island off Georgia, USA.
    - Those animals have a unique lipid physiology that makes them useful in medical and nutritional research.
- The animals can either be kept alive or biological material can be collected to be recovered when needed.

Methods: Live Animals

- The animal preservation used to be a hobby and some zoos existed keeping a few animals.
- Lately, some governments have started paying owners for keeping and breeding an endangered breed, such as the prolific Taihu sheep in China.
- In 1950's, the US Congress passed a law to ensure the conservation of the Texas Longhorn cattle breed as part of the country's living heritage.
  - Today there is a revival of interest among farmers and the breed is no longer in danger.

Methods: Live Animals

- The advantage of keeping live animals is that a breed can respond to the changes in external circumstances progressively and a performance evaluation is possible.
- However, because of high costs, only small populations can be kept; therefore genetic variance declines.

Inbreeding and Genetic Drift

- It was clearly shown by Sewall Wright in the 1930s that inbreeding depression causes fitness problems and extinction in small, isolated populations.
- In addition, danger of losing a unique herd due to disease is high, especially in poultry.

Minimum breeding unit size

- Table in the next slide shows the minimum size of a breeding unit and number of animals that should be replaced to keep inbreeding levels to about 2 % a year.
- The main strategy used to achieve a maximum genetic variation in most conservation programs is to set up a breeding system that maximizes the effective population size.
- These kinds of programs have been shown using Hungarian Grey Cattle and using British Milksheep, to be effective in maintaining genetic variation at least when the populations were in a growth phase.

Minimum number of animals required for conservation by management

	Cattle		Sheep		Pigs		Poultry	
	Male	Female	Male	Female	Male	Female	Male	Female
Size of breeding unit	10	26	22	60	44	44	72	72
No. of breeding animals entering/ year	10	5	22	12	44	18	72	72

Smith, C. (1984): Estimated costs of genetic conservation in farm animals. – FAO Animal Production and Health Paper, 44(1):21-30.

Sample size/Herd size

- A rapid population growth can maintain genetic variability even in the presence of intensive selection.
- However, when breeds with less than 100 females and a few males are taken into consideration, keeping the variance at a high level can be a serious problem.
- Herd size increase = cost increase

Population size

- A random change by genetic drift may override all changes arising for other reasons in small populations.
- Keeping the family variance at a low level (equalizing the family size) is recommended in captive breeding programs
  - Increases the effective population size
  - Reduces inbreeding
  - Slows down the loss of genetic variation

Population size

- In mountain sheep, populations with fewer than 50 animals were subject to rapid extinction within 50 years.
- Six populations – ranging in size from 8-46 – persisted for 34 or more years in Arizona.

Maintaining Genetic Diversity and Factor Analysis

- A breed, which is in danger of extinction, may possess characteristics that could be useful in the future.
  - Disease resistance
  - Adaptation to stressful environments
    - Drought
    - Temperature stress
    - Anything preparing future animals to eat novel types of feed and crop or industrial byproducts.
- However, it is very hard to determine exactly what those attributes might be.

Maintaining Genetic Diversity and Factor Analysis

- Therefore, we may need to keep lines with different attributes with the ones that are not economically valuable today.
- The various lines of live animals can adapt to environmental changes in time and may prove to be more useful than storage of biological material cryogenically.
- Keeping live animals may prove beneficial because they can be used immediately without complicated laboratory techniques of biotechnology.

Maintaining Genetic Diversity and Factor Analysis

- The lines to be kept can be determined using factor analysis.
- By defining covariance relationships among many variables and by describing those in terms of some underlying but unobservable random quantities called factors, one could separate all the animals into well defined-groups.
- This enables the owner of the herd to keep different lines of animals for the future.

Maintaining Genetic Diversity and Factor Analysis

- Animals that are resistant to specific diseases or perform better in various environments can be obtained using these lines.
- The idea in using factor analysis is that one can make groups of animals according to an underlying factor.
- This can be e.g. a disease resistance gene that gives way to a group of animals that are resistant to hunger, or bad nutrition.

Maintaining Genetic Diversity and Factor Analysis

- This can be an enzyme or protein or some other biological factor that makes the animals, for example, behave calmly and also induces higher milk production.
- Whatever the underlying factor is, grouping animals using factor analysis may lead to a better separation, increasing the overall variance.
- Keeping different lines of animals will cause some genes to be fixed and some genes to be lost due to genetic drift.

Maintaining Genetic Diversity and Factor Analysis

- The subpopulations will have smaller variances, but the original variance will be restored when all subpopulations are merged back together, assuming that all circumstances are optimal and there is no selection, migration or mutation.
- This may be a good way to keep the original variance intact since keeping the whole herd together is costly and results in mating of relatives, which increases inbreeding.

Maintaining Genetic Diversity and Factor Analysis

- Spearman invented the common factor analysis.
- Usually, the term factor is a source of confusion in factor analysis.
- It refers to a hypothetical, unobservable variable, as in the phrase common factor.
- In this sense, factor analysis must be distinguished from component analysis since a component is an observable linear combination.

Maintaining Genetic Diversity and Factor Analysis

- A common factor is an unobservable, hypothetical variable that contributes to the variance of at least two of the observed variables.
- The unqualified term “factor” often refers to a common factor.
- A unique factor is an unobservable, hypothetical variable that contributes to the variance of only one of the observed variables.
- The model for common factor analysis posits one unique factor for each observed variable

Maintaining Genetic Diversity and Factor Analysis

- Animals kept for conservation can be used in the future to overcome possible selection limits within the present breeding populations and within the prevailing environment.
- Theory on selection limits and on experimental results with laboratory animals indicates:
  - Animal breeders have to face the hazard that the response to selection "will cease sooner or later, after a continuous decline in magnitude"

Cryogenic Storage

- A variety of cells can be kept using cryogenic methods .
- Living material is stored at –196 Celsius in a liquid nitrogen tank and the length of storage time seems to be indefinite.
- Most disadvantages that apply to live animal preservation can be avoided by storage of frozen cells at lower costs.

Cryogenic Storage

- Next slide has a table to compare the costs of the methods.
- Techniques of cryogenic storage can be summarized as deep-freezing of sperm and oocytes and deep-freezing of embryos
  - Storage of DNA is useless at this point but it may be used in the future.

Estimated costs of stock conservation for different species

	Cattle	Sheep	Poultry
Maintaining a breeding stock (per year) (£)	5 000	3 000	3 000
Frozen semen from 25 sires (£)	9 000	9 000	11 000
Frozen embryos (625 stored) (£)	75 000	50 000	Not possible

http://www.fao.org/docrep/010/ah808e/AH808E03.htm

Estimated costs of stock conservation

- The previous table makes many assumptions depending on level and current cost of the technologies, individual costs and on the levels of inbreeding set by the different methods.
- Collection of frozen embryos is still comparatively expensive, and even collection of ample frozen semen incurs appreciable costs.

Estimated costs of stock conservation

- However these costs are only incurred once, and storage costs are low.
- Maintaining a live breeding stock costs least to start with, but the costs are continually incurred and accumulate over time.
- For periods of over five years, semen storage becomes the cheapest form of conservation.

Sperm and Oocytes

- Deep-freezing semen is possible in all domestic animals, including poultry.
- The techniques are well known.
  - One difficulty is that a relatively complex breeding system is needed to regenerate a purebred population from semen alone.
- At least 5 generations of backcrossing are required to achieve 97 % genes of the rare breed.
- Inbreeding and genetic drift has to be avoided which can be done by using a rotational breeding system.

Sperm and Oocytes

- 25 sires per breed are needed to prevent inbreeding when males are used rotationally on each other's daughters.
- In vitro fertilization (IVF) is already proving to be a powerful tool for rescuing gametes (sperm and oocytes) directly from gonads after death.
  - IVF is a process by which an egg is fertilized by sperm outside the body: in vitro ("in glass").

Sperm and Oocytes

- Manipulation of spermatozoa provides opportunities for the predetermination of sex of resulting offspring, the introduction of foreign DNA into oocytes and formation of transgenic individuals.
- The cryopreservation of oocytes of most animal species remains a challenge due to their complex structure.
- Using these techniques and new ones, one can control the structure of a newborn herd in the future and direct it to the needs of that future time.

Embryos

- Cryopreservation of mammalian embryos has been successfully used in cows, sheep, goats and horses.
- The entire genetic information is stored in a single diploid embryo and no complicated backcrossing programs are necessary. Once the embryos are obtained -which can be done non-surgically now- storage costs become very low.

Embryos

- On one hand, transportation of embryos can reduce/eliminate the need for shipping live animals.
- On the other hand, efficient worldwide movement of germ plasm requires established cryobanks.
- Traditional approaches of superovulation and non-surgical embryo recovery have been hampered in non-domestic ungulate species
  - by inconsistent responses to commercially available gonadotropin preparations; requiring IVF (in vitro fertilization) which is a more expensive procedure than the non-surgical method.

Storage of DNA

- If a breed becomes extinct, one can bring the stored DNA into the active gene pool of the species by insertion of DNA into the embryos of another breed of the same species.
  - This is not feasible as of now.
- DNA is a chemical and not viewed as biological material by animal health and quarantine authorities, so there should not be any problems with international transportation.
- The costs of DNA collection is lower than collecting semen, embryos etc. since this is possible simply by taking some blood samples.

Storage of DNA

- Conservation of rare breeds or vanishing species is necessary. It is clear that one day humans will need those animals.
- In a group of bacteria, some individuals carry disease resistance genes in their plasmids.
- Those individuals are heavier and slower than the others and they require more energy.

Storage of DNA

- However, the other bacteria in the group do not leave behind those who carry the resistance genes.
- In case of a new disease, the bacterium carrying the resistance gene for that specific disease passes along the gene to the others and thus, the whole group is saved.
- In conclusion, it is necessary to keep the animals, which may not be useful today, but can be beneficial in the future.

Conclusion

- Among all of the conservation methods, the easiest and cheapest one is taking and storing DNA/cell samples.
- Although wild animals are meant to be kept alive continuously, rare livestock breeds can be kept as DNA/cell and regenerated when needed.
- In the near future, it may be possible to generate a herd from one cell using the cloning technique (first one animal from one cell and then a herd from that animal’s cells).

Conclusion

- Keeping different lines of animals requires extension of resources, not only for the costs of these animals, but also on management and monitoring the attributes.
- Various kinds of animals should be kept to make sure that the gene pool is available when needed, and keeping different lines can be accomplished by using factor analysis to separate the animals into different lines.
- Live animals can adapt to environmental changes in time and may prove to be more useful though keeping live animals may cost more than cryogenic storage of biological material.

International Union for Conservation of Nature