

# Veterinary Student Scholar

**Assessing admixture between historically isolated mitochondrial DNA lineages in Bornean and Sumatran orang-utans (*Pongo* spp.) in North American zoos**

Alyssa Karklus  
University of Wisconsin School of Veterinary Medicine  
Class of 2020

with

Dr. Graham L. Banes, Assistant Professor, Department of Surgical Sciences, SVM  
Director, Molecular Ecology and Evolution Laboratory at Henry Vilas Zoo

and

Dr. Kurt Sladky, Clinical Associate Professor, Department of Surgical Sciences, SVM

## Background and Significance

Orang-utans include two species found on the islands of Borneo (*Pongo pygmaeus*) and Sumatra (*P. abelii*), which diverged around 400,000 years ago (Locke *et al.*, 2011). The Bornean orang-utan is further divided into multiple geographically and reproductively isolated subpopulations, with three subspecies currently recognized as having shared a common ancestor around 176,000 years ago. Fewer than 15,000 orang-utans are thought to survive on Sumatra (Wich *et al.*, 2016), while the population of Bornean orang-utans, currently numbering 105,000 individuals, is projected to decline by more than 57,000 before the year 2025 (Ancrenaz *et al.*, 2016).

In the face of declining wild populations, the need to conserve healthy and genetically viable populations of orang-utans in zoos is becoming increasingly important. Many zoos across North America participate in captive breeding programs with the aims to maintain healthy captive populations and to contribute to the conservation of wild populations. Typically, such *ex-situ* conservation involves interbreeding the least related individuals, thus reducing the risk of “inbreeding depression” and augmenting genetic diversity (Ballou and Lacy, 1995). However, the opposite phenomenon, “outbreeding depression,” may have potentially negative effects on a population's overall fitness. Hybridization of distinct populations or subspecies, particularly if they occupy different habitat types or have been isolated for more than 500 years, has been linked to equal or greater detriments than inbreeding depression, including developmental, genetic, and other abnormalities (Banes *et al.*, 2016).

Orang-utans are at particular risk of outbreeding depression, both in the wild and in zoos. In an effort to support dwindling wild populations, sanctuaries of orphaned and displaced orang-utans have been established in Borneo and Sumatra, with the ultimate goal of reintroducing individual orang-utans to the wild. Genetic analyses have determined that certain reintroductions from these sanctuaries have resulted in hybridized and introgressed offspring. Notably, some of the hybridized and introgressed individuals have exhibited poor reproductive success and overall ill health; however, the full effects of the admixture remain unclear (Banes *et al.*, 2016). Orang-utans in captivity may represent a similar situation. In the 1920s, orang-utans were collected for exhibition in zoos from across the species' natural range, and therefore may have been indiscriminately hybridized through captive breeding (Elder, 2016). However, the extent of hybridization and introgression among Bornean orang-utans in zoos is yet to be fully characterized. This information is an essential precursor to investigating the effects of such introgression on the health and viability of this critically endangered population. If interbreeding distinct orang-utan subspecies can be linked to reduced fitness or reproductive success, it may be necessary to develop new and independent breeding programs for orang-utans in zoos worldwide, in order to preserve the health of both captive and wild populations. Conversely, if no ill effects are observed as a result of hybridization, this could simplify the procedure for reintroducing more than 1,500 orphaned and displaced orang-utans housed in rehabilitation centers on Borneo and Sumatra (Banes *et al.*, 2016).

## Aims and Hypothesis

I propose to determine the ancestral maternal origins of all 219 orang-utans in the North American zoo population, housed in 53 institutions in the United States, Canada, and Mexico. I will generate mitochondrial DNA sequences from each matriline and compare these with published sequences from orang-utans of known geographic origin. By inferring the subspecies or subpopulation of wild-caught founder individuals, I will assess the extent of hybridization and introgression in the captive orang-utan population over the last century. I hypothesize that orang-utans in North American zoos will derive ancestrally from all three recognized subspecies and from all recognized genetically distinct populations.

## Brief Description of Approaches and Methodologies

Samples from all orang-utans in North American zoos have been collected by my faculty advisor and are housed in the Molecular Ecology and Evolution Laboratory at Henry Vilas Zoo, where I will be based for the duration of the project. I will extract genomic DNA from these samples and design a protocol to amplify the complete mitochondrial DNA control region from at least one orang-utan of each matriline, using the Polymerase Chain Reaction (PCR) (Banes and Galdikas, 2016). The results of the PCR will be visualized on agarose gels and the PCR products will be prepared for sequencing. Under the direction of my faculty advisor, I will become familiar with evaluating the quality of DNA sequences via chromatograms, and using this information, inferring and

collapsing haplotypes. Then, by aligning haplotypes and inferring Bayesian phylogenetic trees, I will assign DNA sequences to populations of known origin.

Complete kinship and pedigree data for orang-utans is available in the form of the International Orangutan Studbook (Elder, 2016) and the *Pongo* database maintained by Dr Banes. By aligning my newly generated sequences with those from orang-utans of known geographic origin, I will identify the ancestral origins of orang-utans in North American zoos. Using *Pongo* and the studbook, I will trace the extent of hybridization and introgression over the past century. This information could serve as preliminary data to influence captive breeding programs and to assess the potential for outbreeding depression within captive orang-utan populations.

### **Role of the Student and Qualifications of the Mentor**

During the duration of this project, I anticipate developing skills in many key areas of biological research. The laboratory work will provide basic transferable research skills in molecular genetics. I will develop experience in the operation and maintenance of a genetics laboratory and become familiar with biosafety levels and working with non-human primate samples. Moreover, I will gain familiarity with the cost of laboratory research and genetic analysis and learn how to manage a research budget. I will also gain a greater understanding of research ethics and procedures, and develop skills in scientific writing. In particular, I will co-author a manuscript for submission to a peer-reviewed journal, and will assist in writing grants to expand this study in future. As my study will likely result in pilot data that can be used to prepare a larger, full-genome-wide research program, I will be involved in determining future directions for longer-term study.

My primary mentor, Dr. Graham Banes, is the director of the Molecular Ecology and Evolution Laboratory at the Henry Vilas Zoo in Madison, WI, as well as an Assistant Professor at the University of Wisconsin School of Veterinary Medicine (SVM). The Zoo is located within 2 miles of the SVM. His research focuses on the use of molecular genetics to the study and conservation of wildlife, including orang-utans. He currently leads *The Orangutan Conservation Genetics Project*, a partner program of the World Association of Zoos and Aquariums, and curates one of the largest biomaterials collections for any critically endangered mammal: more than 3,000 samples from wild, zoo-housed and ex-captive orang-utans were collected over 7 years.

### **Works Cited**

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February 28, 2017

Morris Animal Foundation  
Suite 17a  
720 S. Colorado Blvd.  
Denver, CO 80246

RE: Application of Ms. Alyssa Karklus for MAF Student Scholar Award

The Research Committee has identified Ms. Alyssa Karklus as the 2017 University of Wisconsin nominee for an MAF Student Scholar Award. This letter is to confirm that Ms. Karklus is in good academic standing in the veterinary curriculum, has identified excellent mentors in Dr. Graham L. Banes and Dr. Kurt Sladky, and that a letter of support for Ms. Karklus' application authored by Drs. Banes and Sladky is included with the application materials.

Little or no information is available regarding the genetic diversity of orang-utans, and wild populations of orang-utans are continuing to decrease in numbers. Ms. Karklus' project will take advantage of the unique availability of DNA samples from the entire population of orang-utans in North America zoos to generate novel data that will be extremely valuable in the ongoing effort to sustain orang-utans in the wild and zoos. The Henry Vilas Zoo is in close proximity to the School of Veterinary Medicine, and Dr. Banes and Dr. Sladky have an excellent collaborative relationship that will support Alyssa's training and completion of the proposed research.

If further information is needed to consider her application, please do not hesitate to contact me.

Sincerely,

Dale E. Bjorling, DVM, MS  
Associate Dean for Research and Graduate Training

**School of Veterinary Medicine • Office of Research & Graduate Training**

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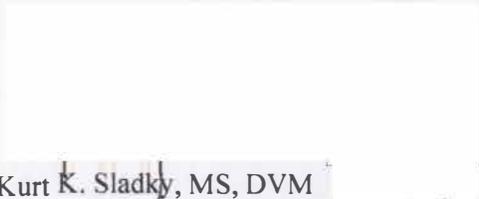
February 17, 2017

Morris Animal Foundation  
Veterinary Student Scholars  
10200 East Girard Ave., Suite B430  
Denver, CO 80231

Dear Morris Animal Foundation Veterinary Student Scholar Program,

We are writing to enthusiastically support Alyssa Karklus' application for the Morris Animal Foundation Veterinary Student Scholar Program. Alyssa is a first-year veterinary student at the University of Wisconsin School of Veterinary Medicine with an interest in pursuing a career in Zoological Medicine. She has several unique experiences working with zoo and wildlife species. In her proposed research project, Alyssa will determine the ancestral maternal origins of orang-utans in the North American zoo population. The results of her study will serve as preliminary data for future research on the potential for outbreeding depression within captive orang-utan populations, and may influence orang-utan pairings within captive breeding programs. During her project, Dr. Graham Banes will serve as Alyssa's primary mentor and Dr. Kurt Sladky will serve as her secondary mentor. For Alyssa, this project will provide excellent laboratory training in molecular genetics and experience collecting and analyzing biological data; developing manuscript preparation and grant writing skills; working with primate biological samples and gaining an appreciation for the benefits of, and ethics associated with, endangered species research; and learning to manage a research project budget.

We have read her proposal and agree to supervise the project as described. We understand that we are responsible for providing any remaining funding for the project, and we assure the Morris Animal Foundation that these funds are available. Thank you very much for your consideration, and please do not hesitate to contact us with any further questions or concerns.

  
Kurt K. Sladky, MS, DVM

  
Dr. Graham L. Banes, PhD

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**Morris Animal Foundation**  
**Animal Involvement Justification**

(From the proposal guidelines, single-spaced, no page limit)

**Morris Animal Foundation (MAF) is dedicated to funding scientifically sound, relevant and humane studies that specifically address the health and well-being of animals. All studies receiving funding must follow MAF's Health Study Policy for Animals Involved in Research (adopted October 18, 2008), which was written to ensure that each and every animal involved in a MAF funded health study receives excellent, compassionate care throughout the study. MAF shall not fund health studies which require euthanasia as an endpoint or the induction of disease or injury, unless the nature of the disease or condition to be studied is of such significance for improving animal health that such means are justified, and that meaningful information can be obtained in no other way. Furthermore, MAF will not fund any study that induces or allows pain or distress unless such pain or distress can be controlled by appropriate anesthetic, analgesic, tranquilizing drugs, or nursing care. [Click here](#) for the full Health Study Policy.**

A. If this study does not involve live animals please indicate here by N/A: N/A

B. Does this study involve biological samples, tissues, etc.? Yes

**If yes**, describe in detail what samples will be used and where & how they will be (or were) acquired. Note: Morris Animal Foundation reserves the right to request a copy of the Institutional Animal Care and Use Committee (IACUC) application/approval and other relevant applications/approvals (e.g., wildlife permit) covering the original collection of samples, including archived samples. MAF reserves the right to request IACUC (or equivalent) review and approval for any Foundation study ***regardless of the Institution's requirements***. This would include the use of archived samples as well as clinical trials.

Genetic samples, comprising blood products, feces, shed hair, and oral (saliva) swabs, were collected over a seven-year period from 53 accredited zoological institutions in the United States. All samples were collected non-invasively or during routine medical procedures with ethics approval from each institution's Animal Care and Use committees. Sample collection was also approved by letter of recommendation from the Orangutan Species Survival Plan, and conformed to the additional ethical principles of both the American Society of Primatologists and the International Primatological Society.

C. If this study involves live animals, succinctly address the following: (please restate the questions and directives). This study does not involve live animals, only biological samples.

1. What species will be studied?

2. State the status of your IACUC application/approval. All recipients of MAF funding will be required to submit the entire IACUC protocol and document. A copy of the IACUC approval should not be included with the application, but it is required before funding can be awarded.

3. List the USDA category for pain and distress (B, C, D, E):

Note: Any study beyond category C will require review by MAF's Animal Welfare Advisory Board (AWAB). In general MAF does not fund studies beyond category C (category D studies will only be considered if they conform with MAF's Health Study Policy, category E studies will not be considered).

4. Does this proposal involve client-owned animals?

If yes, the protocol for client-owned animals must be approved by the appropriate peer review committee before the project is funded. *If this proposal involves client-owned animals, an informed client consent form must be submitted with this proposal. For a suggested list of items to be considered in an informed client consent form, [click here](#).*

5. Explain how animals will be acquired (e.g., client-owned, USDA licensed breeder, institutional "herds" or "colonies") and verify that the animals are suitable for the study (e.g., have no physiologic, physical or

pharmacologic issues that would interfere with results)

6. How many animals will be used?

a. Summarize numerical justification

7. Does this study induce disease, injury, pain or distress in animals?

Note: any study requiring the induction of disease, injury, pain, or distress will have an additional evaluation by MAF's AWAB.

If yes,

a. Defend the necessity of experimental design

b. Explain how pain and/or distress will be controlled

c. Justify that no alternative, including clinical studies, can be used to accomplish study objectives and the disease/condition to be studied is of such significance for improving the health of the species.

8. Explain the environment and housing conditions (quality of life) in which the animals will live (address species-appropriate exercise, enrichment, socialization, veterinary care, etc.)

9. What will happen to the animals upon completion of the study?

If adoption, explain the adoption process. Provide assurance that whenever possible and when in the animal's best interest, investigators shall make companion animals available for adoption at the end of the study or return the animals to the owner/responsible agency in an environment that promotes animal welfare and excellent quality of life.

10. If euthanasia, provide the following additional information (note: any study requiring euthanasia as an endpoint will have an additional evaluation by a MAF's AWAB.

i. Total number that will be euthanized and justification for numbers

ii. Method of euthanasia

iii. Justification that no alternatives can be used to accomplish study goal(s) and that the disease/condition to be studied is of such significance for improving the health of the species that a terminal endpoint is deemed necessary.

iv. Reason for euthanasia in lay language (this wording may be shared with staff, donors and media)

v. Provide objective criteria for determining when euthanasia is appropriate or necessary (note: Morris Animal Foundation wants assurance that an animal will not be allowed to suffer and that monitoring for pain and suffering is adequate)

Note: Morris Animal Foundation does not consider the use of CO<sub>2</sub> alone to be an appropriate method of euthanasia

**Please note:**

1. If an animal is used in an invasive study, MAF may require that a guarantee is provided, through principal investigator and institutional signatures that the animal will not participate in any future invasive study or procedure
2. MAF does not allow inclusion of ancillary data in MAF funded research that includes animal use protocols not in agreement with our Health Study Policy, even if it is obtained using other funding sources.
3. Morris Animal Foundation considers euthanasia acceptable when an animal develops unanticipated illness or injury that results in pain and suffering that cannot be alleviated with standard veterinary interventions.