



National Leadership Grants for Museums

Sample Application MG-30-15-0102-15
Project Category: Collections Stewardship

University of Wisconsin - Milwaukee

Amount awarded by IMLS: \$454,163

Attached are the following components excerpted from the original application.

- Abstract
- Narrative
- Schedule of Completion

Please note that the instructions for preparing narratives for FY2016 applications differ from those that guided the preparation of FY2014 and FY2015 applications. Most obviously, the names of the three narrative sections and the order in which they appear have changed. Be sure to use the narrative instructions in the FY2016 Notice of Funding Opportunity for the grant program and project category to which you are applying.

Abstract

The University of Wisconsin – Milwaukee, in collaboration with San Diego Zoo Global and an Implementation Team of zoo and aquarium Population Biologists, proposes to lead a research project to improve the collaborative management of living collections maintained by Association of Zoos and Aquariums (AZA) facilities. Nearly 500 cooperatively managed Species Survival Plan® (SSP) programs provide a framework for zoos and aquariums across the US to collectively breed and maintain priority collection species. Unfortunately, the AZA community is facing a species “sustainability crisis”, and it is now commonly accepted that numerous priority species in these collections cannot be maintained unless new management strategies are adopted. This project proposes to address one significant aspect of the species sustainability crisis – maintaining genetic diversity in living collections – by developing an easily accessible workflow and infrastructure to improve genetic diversity retention for SSPs with incomplete or poorly known pedigrees.

SSP breeding strategies rely on accurate pedigrees to preferentially select the most genetically valuable animals for retaining genetic diversity. Unfortunately, incomplete or poorly known pedigrees are systemic throughout SSPs and hamper both effective breeding pair selection and the long-term sustainability of these species in AZA collections. Pedigree information is currently available for 361 SSP populations; 78% of these species have some amount of unknown pedigree and 31% have less than half of their pedigrees known. Next-generation sequencing methods for DNA marker discovery have the potential to revolutionize the genetic management of these SSPs by providing very accurate empirical estimates of the kinships between animals. However, this methodology has yet to be adopted by AZA breeding programs, primarily due to a general lack of expertise in molecular genetics within the AZA community.

The goal of the proposed collaborative research project is to develop, test, and deploy the necessary infrastructure (protocols, software programs, training workshops, etc.) for SSPs to effectively integrate DNA data into breeding pair selection strategies, to ultimately improve the long term retention of genetic diversity in these collections. Project activities will be completed over three phases: 1) research and development of protocols, methods, and software to integrate DNA data into breeding pair selection; 2) validation and refinement of the accessibility and efficacy of the infrastructure through partnerships with representative SSPs to serve as case studies; and 3) implementation of infrastructure and evaluation of project outcomes, through a training workshop, quantification of pedigree improvement, and end-user surveys.

Three project outcomes are expected and will be evaluated by a number of performance indicators that include summary statistics and “before and after” project surveys. The first outcome is an efficient process for SSPs to integrate DNA data into breeding pair selection for species with poorly known or incomplete pedigrees; no such formal process currently exists. The second outcome is improved pedigrees and breeding pair selections for five SSP species, which will demonstrate the efficacy of the project outputs provided to the AZA community. The third outcome is a change in attitude within the AZA community regarding the incorporation of DNA data into breeding pair selection. Although DNA data have historically been generated for a modest number of SSP species, genetic management benefits have been largely inconsequential and difficult to quantify because effective DNA data integration has been historically ineffective. Clearly measuring and demonstrating the benefits of project results and outputs is thus essential for affecting a positive change in attitude toward the use of DNA data in the genetic management of SSPs. Collectively, project outcomes are expected to result in improved SSP management, through better breeding recommendations, designed to support the long term persistence of AZA’s living collections.

1. Project Justification

The University of Wisconsin – Milwaukee (UWM), in collaboration with San Diego Zoo Global (SDZG) and an Implementation Team of zoo and aquarium Population Biologists, seeks a National Leadership Grant in the Collections Stewardship category to improve the collaborative management of living collections maintained by Association of Zoos and Aquariums (AZA) facilities. AZA currently facilitates the formal management of 474 cooperative Species Survival Plan® (SSP) Programs. SSPs provide a framework for zoos and aquariums across the US to cooperatively breed and maintain priority collection species for exhibit, conservation, and research. In some cases, such as the Guam rail and the Przewalski's horse, SSP programs have been vital for preventing species extinction. However, recent analyses conducted as part of the management of these SSPs suggest that the zoo and aquarium community is facing a crisis in their ability to maintain viable populations of species¹. The proposed research project will address one significant aspect of this sustainability crisis – maintaining genetic diversity in living collections. We will develop an easily accessible infrastructure, including laboratory protocols, analytical tools, and a software module, to effectively integrate DNA data into pedigree-based breeding strategies. The research results and resources provided to the AZA community through this project are expected to result in improved cooperative species management among zoos and aquariums, better breeding recommendations, and greater persistence of living collections.

The Sustainability Crisis: The AZA community now widely recognizes that many species currently maintained across their facilities represent small, genetically depauperate populations that have poor prospects for long term self-sustainability. For example, when populations of cooperatively managed AZA species were evaluated in 2011¹, 40% of populations were in decline ($N_{\text{species}} = 289$), the median population size was only 66 individuals per species across all AZA institutions ($N_{\text{species}} = 428$), and the median number of population founders, a measure of genetic vigor, was just 15 individuals ($N_{\text{species}} = 264$). Numerous challenges to species sustainability have been well-documented by the zoo and aquarium community^{2,3}. Some of the more significant, known challenges include insufficient “space” across AZA institutions to increase the number of individuals per species, impediments to importing animals for bolstering genetic diversity (e.g, cumbersome legislation and permit processes), inadequate transfer of animal husbandry and breeding expertise across generations of animal care staff, a paucity of institutional and community resources for effectively implementing cooperative management, and poor or unknown pedigrees that hinder the process of selecting genetically beneficial breeding pairs. Given the challenges facing species sustainability across the AZA community, it is now commonly accepted that numerous priority species in these collections cannot be maintained over the long term unless new collection management strategies are adopted.

The Importance of Genetic Management: One of the primary factors contributing to the species sustainability crisis in AZA facilities is the inability of SSPs to retain high levels of genetic diversity within their cooperatively managed populations. If a small population is closed to the import of new individuals, like many zoo and aquarium populations, subsequent generations will become more and more related to each other over time. This causes a decline in genetic diversity and an increase in inbreeding across the population, which ultimately has negative effects on individual fitness^{4,5,6}. For example, an inbred individual may die sooner than expected, fail to successfully raise offspring, or be particularly susceptible to disease. This negative correlation between inbreeding and survival has been documented in captive populations, for example in okapi where increased inbreeding results in higher juvenile mortality rates⁷. It is critically important for SSPs to retain high levels of genetic diversity in their populations, so that priority species continue to be available to zoo and aquarium collections in the future.

To maintain genetically viable populations over the long term, animals with the lowest average kinship (i.e., relationship) to the rest of a cooperatively managed population are preferentially selected to breed^{8,9,10}. Breeding strategies designed to minimize kinship across a population are widely accepted and implemented by regional zoo and aquarium associations around the world, because both theoretical and empirical research has demonstrated these strategies are the most effective for maintaining a captive population’s genetic diversity^{8,11}. However, because these breeding strategies rely on accurate pedigrees, incomplete or poorly known population histories hamper the genetic management of hundreds of SSPs. When pedigrees are unknown or incomplete, the best breeding pairs for maintaining genetic diversity cannot be identified and a rapid loss of gene diversity can occur. The problem of unknown pedigrees is systemic throughout AZA’s SSPs Programs. Pedigree information is currently available for 361 of the 474 SSP populations. In 78% of these species there is some amount of unknown pedigree and 31% have less than half of their pedigrees known.

To illustrate the problem, below is the pedigree for jaguar 1003, or “Nabalam” as she is known at her institution. Parents are depicted above each jaguar in the diagram, connected to their offspring by solid lines. Circles represent females and squares represent males. Although the parents (jaguars 986, 862) and grandparents (jaguars 657, 663, 591, 590) of Nabalam were documented, ancestry further back in her pedigree remains unknown. Jaguar 381 and 289 were captive-born animals acquired from undocumented sources by AZA facilities in 1972 and 1965, respectively. Jaguars 405 and 247 were born at AZA facilities in 1969 and 1962, but their parents were never recorded.

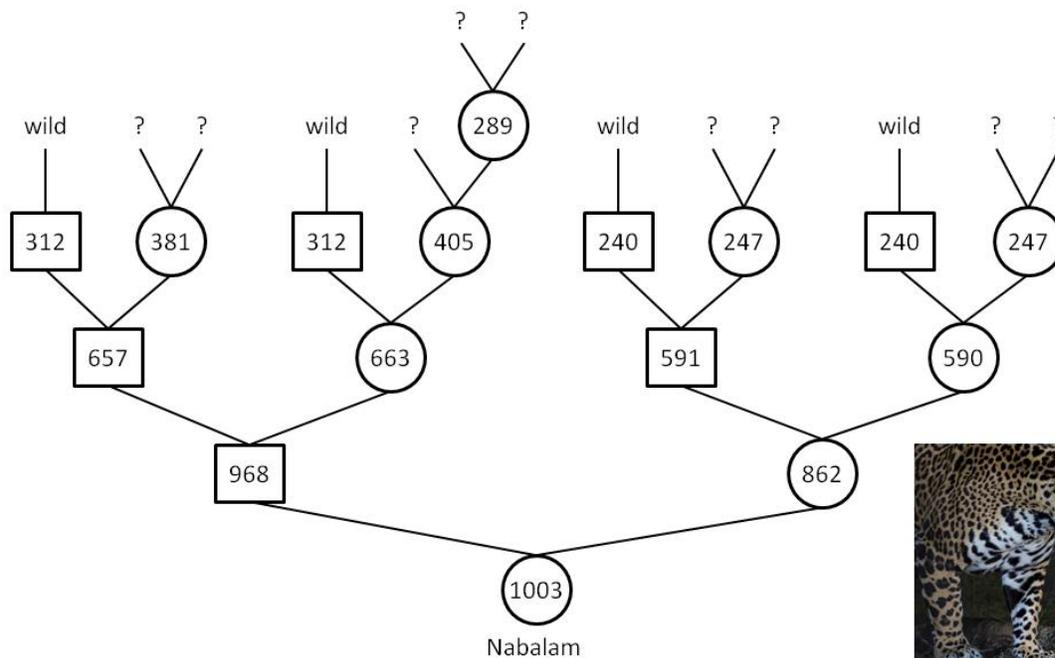


Photo Credit: San Diego Zoo Global

The great-great-grandmother of Nabalam, jaguar 289 or “Ecer”, is particularly complicating because she had 15 cubs at the same AZA facility between 1968 and 1972. Because the paternity of those 15 cubs was never recorded, we do not now know if they were full-siblings from the same father or half-siblings from different fathers. We also do not know if the father(s) of those cubs sired other cubs at other AZA institutions, which would further complicate relationships. In short, because of the uncertainty introduced into Nabalam’s pedigree by Ecer and other ancestors (jaguars 381, 405, 247), we cannot, with any confidence, identify Nabalam’s living relatives. Consequently, Nabalam is excluded from breeding because population biologists cannot identify a genetically appropriate mate for her.

Building Pedigrees with DNA Markers: Next-generation sequencing methods^{12,13,14} have the potential to revolutionize the genetic management of SSPs with incomplete or poorly known pedigrees, because for the first time we can use a large number of DNA markers to calculate very accurate estimates of kinship between animals^{13,15,16}. Restriction site associated DNA (RAD) sequencing, in particular, has proven to be a reliable, taxonomically flexible method for characterizing high density suites of single-nucleotide polymorphisms, or SNPs, that can be used to reliably estimate kinship¹⁷. Unfortunately, this methodology has yet to be adopted by AZA breeding programs, primarily due to a lack of guidance on how to partner with academic or private labs to generate appropriate DNA data, insufficient experience analyzing the immense amount of generated data, and lack of knowledge in how to effectively integrate those data into current breeding pair selection strategies. The proposed collaboration between The University of Wisconsin – Milwaukee and the AZA community will facilitate the application of DNA data to AZA breeding programs by researching and developing an easily accessible process and infrastructure that non-experts in molecular genetics can use to incorporate DNA data into breeding pair selection when pedigrees are poorly known or incomplete. Our program will ultimately improve the long term retention of genetic diversity in SSP Programs.

Project Overview: The proposed project aims to answer three broad research questions. **Question 1:** What are the most effective methods, at each step of data integration, for utilizing DNA data in breeding pair selection? Although project collaborators have identified the general steps required to integrate DNA data into breeding pair selection, there are numerous technical details at each step that must be researched and adapted to SSP management. **Question 2:** What immediate benefits are gained during breeding pair selection if DNA data is incorporated into the process? Although DNA data has been generated for a modest number of SSP species, the benefits to breeding pair selection and long term genetic diversity retention have been largely inconsequential and difficult to quantify because DNA data integration has not been ad hoc and inconsistent. Consequently, to change attitudes within the AZA community regarding the use of DNA data in genetic management, it is essential that the proposed project measure and demonstrate specific benefits to breeding pair selection. **Question 3:** Do the immediate benefits identified for Question 2 persist over time to improve long term retention of genetic diversity? Like Question 2, it is essential that the proposed project demonstrate long term benefits to genetic diversity retention to change attitudes within the AZA community and justify the expense, in terms of funding and resources, for generating DNA data.

By answering the three research questions just described, the proposed project will develop, test, and deploy the necessary infrastructure (protocols, software programs, training workshops, etc.) for SSP Programs to effectively integrate DNA data into breeding pair selection strategies designed to maximally retain a population's genetic diversity. Dr. Emily Latch, an Associate Professor at UWM, has extensively consulted with AZA Population Biologists at SDZG (Drs. Jamie Ivy and Andrea Putnam) throughout the inception of this project, and both sides believe this to be a valuable collaboration that will directly improve the stewardship of AZA's living collections. All project activities will be completed by a Research Team that includes Drs. Latch, Ivy, and Putnam, plus a proposed UWM graduate student and a SDZG post-doctoral researcher. To ensure that this project effectively meets the needs of the AZA community, we have assembled an Implementation Team of AZA Population Biologists who will be involved in all stages of the project. The implementation Team will be composed of biologists who will ultimately utilize the developed infrastructure to integrate DNA data into SSP breeding pair selections (see Supportingdoc2.pdf for letters of support from Implementation Team). By involving the Implementation Team from the outset, the Research Team has been able to design and will be able to implement the project as a strategic investment that maximizes the impact of the project for the AZA community. Training workshops will be designed to transfer the necessary knowledge and tools to AZA Population Biologists, facilitating the advance of captive collections management into the 21st century.

To test and demonstrate the efficacy of the developed infrastructure from start to finish, the Research Team will generate SNP data for five SSP species with poorly known or incomplete pedigrees and then use that data to make improved breeding recommendations. This group of species represents a diverse taxonomic assemblage that will demonstrate the effectiveness of our infrastructure across varied pedigree structures. In total, 89 AZA facilities currently have one or more of these species in their collections and will directly benefit from these improved SSPs.

2. Project Work Plan

Project Goals: The goal of the proposed three-year project is to provide the AZA community with the infrastructure necessary to effectively integrate DNA data into breeding pair selections for SSPs with incomplete or poorly known pedigrees. The expectation is that the resources provided to the AZA community through this project will result in improved collections stewardship, through better breeding recommendations, designed to support the long term persistence of AZA's living collections.

Project Activities: Project activities will be completed over three phases. The first phase of our project will focus on the development of the protocols, methods, and software needed to effectively integrate DNA data into breeding pair selection. Activities during the second phase of the project will focus on refining the accessibility and efficacy of the infrastructure designed during the first phase. Finally, the third phase of the project will comprise implementation of the infrastructure and evaluation of project impacts. Please see the attached Schedule of Completion for a detailed description of when activities will occur.

Phase 1 - Develop Infrastructure to Generate, Analyze, and Employ DNA Data

To allow non-experts in molecular genetics to efficiently integrate DNA data into SSP breeding pair selection, the Research Team will develop a complete, user-friendly infrastructure. This phase of the project will address research Question 1: *What are the most effective methods, at each step of data integration, for utilizing DNA data in breeding pair selection?* The infrastructure will include the following components, each requiring targeted research on the technical details needed to adapt existing methods and protocols to SSP management.

1. Standardized Data Collection: Restriction site associated DNA (RAD) sequencing is currently the academic standard for generating high density suites of single-nucleotide polymorphisms, or SNPs, on non-model species for which genomic resources are lacking^{18,19}. RAD sequencing methodologies, however, vary among labs. To facilitate consistent downstream data analyses and integration of DNA data into breeding recommendations, the Research Team will investigate alternate protocols and formalize a standard methodology that individuals with little or no experience in genetics can use when partnering with universities or labs to generate SNP data. Key features of a standardized methodology will include choice of restriction enzymes used to fragment DNA, the application of discrete barcodes for identifying data from particular individuals, size selection of fragments to be sequenced, and next-generation sequencing platform.

2. Software and Protocol for Data Analyses: The software program most commonly used to analyze SNP data collected from RAD sequencing is Stacks²⁰. Stacks is a freely available software program that is already in use by some members of the Research Team. Because Stacks has been modularly designed to perform several different types of genetic analyses, a modicum of both genetic knowledge and computer programming skills is necessary to efficiently utilize the program for generating SNP data from RAD sequencing. Furthermore, additional software programs are needed both before and after Stacks to fully analyze and integrate SNP data into SSP breeding recommendations.

The Research Team will develop custom software modules to be used before and after Stacks, as well as a detailed Stacks protocol, to create a single, comprehensive data analysis pipeline targeted specifically for AZA Population Biologists. Modules will be created using the Microsoft Visual Studio .NET development tools. Initial modules in the pipeline will prioritize animals for DNA sample collection to maximize the benefit in resolving pedigree uncertainty and prepare RAD sequencing data for analysis in Stacks. The detailed Stacks protocol will include protocols to clean and organize the raw sequence data, create a catalog of SNP markers, and output the SNP data for each individual in the appropriate format. The user-friendly documentation on how to use the software will be created in a similar style to Steve Palumbi's very successful Simple Fool's Guide (<http://sfg.stanford.edu>) developed for a different type of genomic analysis. A final module will calculate SNP-based estimates of kinship using an allele-sharing function already in use by project collaborators.

Modules to be developed:



3. Data Integration into Breeding Pair Selection: The final components of the proposed infrastructure are custom software modules to integrate SNP-based estimates of kinship with known portions of a population's pedigree to produce improved kinships, and provide a kinship file that can be imported directly into the software program PMx for breeding pair selection. PMx is a freely available software program written in the C# programming language²¹; it is a package of demographic and genetic analysis tools to assist with the management of wildlife breeding programs and is the definitive software used for SSP breeding pair selection.

A critical component of the proposed analysis pipeline is a module to integrate SNP-based estimates of kinship with known portions of a population's pedigree. Most SSP populations with poorly or incompletely known pedigrees still have some portion of their pedigrees recorded. Because sampling 100% of currently living animals to estimate all pair-wise kinships with DNA data is both impractical and inefficient, the most effective method of utilizing DNA data to provide improved estimates of kinship is one that also incorporates any known portions of a population's pedigree that may be available²². Thus, the Research Team proposes to generate improved estimates of kinship by using a mathematical optimization algorithm to identify the kinships among a pedigree's "terminal" individuals (i.e., animals with unknown parents) that produce pedigree-based kinships that strongly correlate with those estimated from DNA (see Supportingdoc1.pdf for a more detailed explanation of methods). Although this integration strategy has been previously employed^{22,23}, alternate mathematical optimization algorithms have not been rigorously tested to determine the most effective process. As part of the development of a module to integrate SNP-based estimates of kinship with known portions of a population's pedigree, we propose to use custom computer simulations written in the C++ programming language to test three of the most promising optimization options (an exhaustive search²², simulated annealing²³, and a Bayesian approach). The optimization algorithm that results in the most accurate pedigree-based kinships that best correlate with those estimated from DNA will be incorporated into our analysis pipeline. The final module in the proposed pipeline will produce a matrix of improved kinship values that can be directly imported into PMx and immediately utilized in current breeding pair selection processes.

Phase 2 - Confirm Accessibility and Efficacy of Infrastructure

The Research Team will confirm the accessibility and efficacy of the developed infrastructure to integrate DNA data into SSP breeding pair selection by partnering with a small number of SSPs to generate, analyze, and incorporate DNA data into breeding pair selection (see Supportingdoc2.pdf for letters of SSP support).

The five species selected for the project include Queensland koala, addax, Inca tern, fennec fox, and Eurasian eagle owl. This group of species represents a diverse taxonomic assemblage that will demonstrate the effectiveness of the developed infrastructure across varied pedigree structures. In total, 89 AZA facilities currently have one or more of these species in their collections and will directly benefit from SSPs with more accurate pedigrees and, thus, greater ability to sustain populations.

Queensland koala: Although the contemporary koala pedigree is very well-known, kinships among both historic and more recent imports from Australia remain unclear. Only 39% of the pedigree of the living SSP population can be traced to imports, which can reasonably be assumed unrelated, and not a single living animal can be conclusively traced to wild-caught animals known to be unrelated. A total of 58 koalas have already been sampled, which will resolve the relationships among 44% of the pedigree's terminal individuals.

Addax: Records suggest that addax have been held in AZA collections since 1955, but accurate pedigree records were not maintained for this herd species until much more recently. Furthermore, because a large population of addax exists on private ranches in the US, there have historically been exchanges of animals of unknown origin between collections in the private sector and AZA. The combined effect of these two historic situations is that only 13% of the pedigree of the living SSP population can conclusively be traced to unrelated population founders. The addax, like the koala, is an excellent species to include in this project because 128 animals have already been sampled. Furthermore, SNP data on 48 addax have already been generated through RAD sequencing techniques as part of a pilot study for our proposed project.

Inca tern: Inca terns are a threatened species found off the coast of Peru and Chile, and have been breeding in AZA institutions since the 1960's. Institutional interest in exhibiting Inca terns is high within AZA institutions; however, captive breeding cannot meet the demand because of unknown pedigrees. Currently 72 Inca terns with unknown pedigrees are excluded from breeding, which is 30% of the SSP population. This project would aim to return these birds back into the breeding population through estimating their relatedness to the rest of the population. This would also ease the burden on AZA institutions, which have to manage the excluded birds separately.

Fennec fox: The fennec fox is a popular animal in the US's pet trade and can be legally owned throughout much of the country. As a consequence, many of the foxes in the currently living SSP population are of unknown origin because they were surrendered or confiscated from private owners. Because there are a limited number of fennec fox breeders in the US, the SSP believes that many of the SSP animals that originated in the private sector are likely related. The fennec fox is an excellent species to include in this project because 147 animals have already been sampled, which will resolve the relationships among 100% of the pedigree's terminal individuals.

Eurasian eagle owl: The Eurasian eagle owl is one of the world's largest owl species. This charismatic owl is held by 35 AZA institutions and frequently used for outreach programs. The species is coveted in the private sector, where most breeding has historically occurred. Unfortunately, accurate pedigree

records have been impossible to obtain from these private breeders as birds were transferred into the SSP. Although breeding within the SSP has greatly improved, recommendations are hindered by the unknown pedigree of the population's ancestors. Within the SSP, only 3% of the pedigree is known and extensive pedigree assumptions are needed in order to make breeding recommendations.



Photo Credits: San Diego Zoo Global (koala), Math Knight (addax), Patrick Coin (tern), Yvonne N (fox), and Christopher Michel (owl)

Phase 3 – Implement and Evaluate Infrastructure

The third phase of the proposed project is to implement and evaluate the infrastructure we developed in Phase 1 and tested in Phase 2. This phase of the project will address research Question 2: *What immediate benefits are gained during breeding pair selection if DNA data is incorporated into the process?* and research Question 3: *Do the immediate benefits identified for Question 2 persist over time to improve long term retention of genetic diversity?*

To fully implement the infrastructure, the Research Team proposes to hold a two-day workshop to train AZA Population Biologists (the Implementation Team). Workshop participants will receive an overview of how the standard methodology developed in Phase 1 was used to collect SNP data on the five species utilized for Phase 2. The bulk of the workshop will be a detailed, interactive demonstration of how to use all tools comprising the developed data analysis and integration pipeline. The five species utilized during Phase 2 will provide the necessary input data for this demonstration and associated exercises. The goal of this third phase of the project is to ensure the AZA Population Biologists responsible for SSP breeding recommendations are adequately trained and confident in their future use of the new tools that the project will provide to the AZA community to improve the long term sustainability animal collections.

The Research Team proposes to assess the impact of the project in three ways. First, impact will be assessed by measuring the extent to which the pedigrees of the five species utilized during Phase 2 were improved, as well as the number of living animals that were previously excluded from breeding that can now receive recommendations due to improved pedigrees. These assessments will provide data to identify the immediate benefits gained during breeding pair selection when DNA data is incorporated into the process (Question 2). The second impact assessment will be the difference in gene diversity (expected heterozygosity) under two sets of population projections for the five species utilized during Phase 2; gene diversity retained at 100 years will be compared for each species both before and after DNA data was used to improve the pedigrees. This assessment will provide data to quantify the long term impact improving pedigrees with DNA data has on genetic diversity retention (Question 3). Finally, to measure the impact of the project within the AZA community, the Research Team will conduct “before and after” surveys of both the AZA Population Biologists and SSP Coordinators to empirically measure changes in attitude toward investing in DNA data and the future likelihood of incorporating DNA data into SSP breeding pair selection. If the AZA community is more likely to incorporate DNA data into SSP breeding pair selection after being provided with the project's outputs, the project will be considered a success.

Project Resources: The project will be coordinated by **Dr. Emily Latch**, Associate Professor at University of Wisconsin – Milwaukee. Latch's research focuses on the genetic consequences of wildlife management, and

she has worked extensively with a number of iconic North American species that include mule deer, wild turkeys, river otters, fishers, American badgers, and American bison. Some of her most prominent projects have involved the genetic analyses of extremely large datasets, comprising thousands of animals from hundreds of locations. In addition, the majority of Latch's projects have involved collaboration with federal agencies (e.g., US Fish & Wildlife Service), and Latch is experienced with facilitating such endeavors. Latch will supervise a **proposed graduate student**, who will be responsible for formalizing a standard methodology for generating SNP data (Phase 1), assisting with analysis software and protocol development (Phase 1), generating SNP data (Phase 2), and assisting with the training workshop (Phase 3). Latch's genetics laboratory is fully equipped for all project activities related to SNP data collection and analyses.

Primary collaborators within the AZA community are Population Biologists **Dr. Jamie Ivy** and **Dr. Andrea Putnam**. Ivy is a Senior Population Biologist for San Diego Zoo Global (SDZG), while Putnam is a Population Biologist for the California Association of Zoos and Aquariums (CAZA); both are housed at SDZG facilities in California. Ivy and Putnam have extensive experience working with AZA's living collections, and combined they develop breeding recommendations for 83 SSPs. Ivy and Putnam also have PhDs in molecular genetics and notable publishing histories in both genetic management of captive populations and integrating DNA data into breeding pair selection. Ivy and Putnam will supervise a **proposed post-doctoral researcher**, based with them at SDZG facilities, to develop custom software tools (Phase 1), organize the training workshop (Phase 3), and administer project evaluation surveys (Phase 3). Ivy and Putnam are both proficient in a number of computer programming languages and have extensive experience in the construction of custom simulations and software, making SDZG the logical choice to base the post-doctoral researcher.

To maximize the impacts of this project within the AZA community, the Research Team will engage an **Implementation Team** comprised of targeted end-users. The Implementation Team will include staff from AZA's Population Management Center (PMC) and PMC Adjunct Population Biologists working for a variety of AZA institutions across the US. The Implementation Team will receive biannual project reports and be invited to participate in biannual web meetings or conference calls, to allow ample opportunities for feedback throughout the duration of the project. Discussion with the Implementation Team will be used to guide and refocus project activities after each biannual update. At the end of the project, the Implementation Team will be fully trained on the use of the new infrastructure and tools that will be provided to the AZA community.

This project represents an innovative collaboration between academia and the AZA community, and the personnel involved lend the project a high probability of success. Latch and Ivy are established collaborators, who most recently were awarded a grant from the Department of the Interior to use custom computer simulations to test the genetic impact of different culling strategies for bison herds managed by the US Fish & Wildlife Service. That project also utilized both DNA data and custom computer software to ultimately improve the long term retention of genetic diversity in wildlife populations, and demonstrated an ability to successfully integrate population management concepts from the zoo community with academic resources.

The \$454,163 project cost includes partial salary and benefits for Latch and the graduate student at UWM, as well as 100% of salary and benefits for the three-year post-doctoral researcher at SDZG. Other significant budgeted items include computing resources, travel funds for project-related meetings, and funds to collect DNA data on the project's five target species. The training workshop will be held in conjunction with the AZA Midyear Conference in 2018 and is not part of the project's budget. Similarly, UWM will pay the bulk of the salary and benefits for Latch and the graduate student during the three-year project period.

Dissemination: Project protocols and software modules developed to incorporate DNA data into breeding pair selection for SSPs with incomplete or poorly known pedigrees will be distributed to all AZA Population Biologists and freely available for download on Latch's Applied Evolutionary Ecology website. Project results will be disseminated to the wider AZA community through presentations at AZA conferences, an article in AZA's *Connect* magazine, and at least one of the anticipated publications will be submitted to the journal *Zoo Biology*. The Research Team also anticipates producing additional publications detailing SNP characterization and the framework for incorporating genomic data into pedigrees, which will be published in peer-reviewed scientific journals such as *Molecular Ecology* and *Evolutionary Applications*. These publications will serve to advance the science of live collections management and draw attention to the important work being done in this field. Finally, management impacts on the project's five target species will be discussed and integrated into AZA Breeding & Transfer Plan publications that are distributed to all AZA facilities holding those species.

3. Project Results

The goal of the proposed research project is to develop infrastructure and resources to address the need of improved genetic management in many of AZA's almost 500 Animal Programs. Three outcomes are expected. The first outcome is an efficient process for SSPs to integrate DNA data into breeding pair selection for species with poorly known or incomplete pedigrees; no such formal process currently exists. The second outcome is improved pedigrees and breeding pair selections for five SSP species with poorly known pedigrees, which will demonstrate the efficacy of the project outputs provided to the AZA community. The third outcome is a change in attitude within the AZA community regarding the incorporation of DNA data into breeding pair selection. Because historic attempts at utilizing DNA data for breeding pair selection have been largely unsuccessful within the AZA community, the dissemination of an effective infrastructure that can be used by non-experts in molecular genetics is expected to result in a positive change in attitude toward the use of DNA data in the genetic management of SSPs. In addition to the direct outcomes expected, our project is designed to support STEM initiatives by advancing learning and supporting the acquisition of knowledge in cutting-edge STEM disciplines including genetics and bioinformatics. These initiatives will be supported in the community of professionals and mid-career managers, whose opportunities for lifelong learning are often limited, to help them learn and employ 21st century skills to advance the conservation field.

End-User Results: AZA Population Biologists will benefit from this project through an increased knowledge of how and when to use DNA data to resolve the problem of unknown pedigrees, as well as by acquiring new tools designed specifically for non-experts to assist in generating DNA data and incorporating it into breeding pair selections. To evaluate project outcomes from an end-user perspective, the Research Team will measure a series of performance indicators through online "before and after" project surveys. Survey questions will be designed to measure changes in the confidence and skills of AZA Population Biologists to integrate DNA data into breeding pair selections. Because the AZA Population Biologists are the project's Implementation Team, their participation throughout infrastructure development will be critical to the success of the project and its long term benefits and sustained use. Their feedback during the funding period will allow us to adjust the methodology guides to ensure they are user-friendly and maximize the adoption of resources developed through the project. If the performance indicators evaluated through project surveys indicate that AZA Population Biologists are more confident in their abilities to incorporate DNA data into SSP breeding pair selection after being provided with the project's outputs, the project will be considered a success.

AZA Animal Program Results: The Research Team will demonstrate the project's proof of concept by applying the newly developed tools to five SSP Programs whose unknown pedigrees currently hamper breeding recommendations, and hence those Programs' long term sustainability within AZA institutions. During the

funding cycle, the Research Team expects to resolve unknown pedigrees through accurately identifying relationships among individuals in the project's five target species. The Research Team will measure the following performance indicators to evaluate project outcomes from a SSP perspective: the extent to which the pedigrees of the project's five target species were improved, the number of living animals that were previously excluded from breeding that can now receive recommendations due to improved pedigrees, and the difference in gene diversity projected to be retained at 100 years for each of the project's five target species both before and after DNA data was used to improve pedigrees. Each of these performance indicators can be evaluated through currently available population management software. Increases in the proportion of pedigrees known, animals available for breeding recommendations, and long-term retention of gene diversity will all indicate project success.

Sustaining Results: To sustain project outcomes beyond the three year funding period, the Research Team will establish an AZA Scientific Advisory Group (SAG) comprised of AZA members with genetic expertise. SAGs represent, in part, the AZA community's commitment to increasing the use of science in the management and care of its animal collections. A newly initiated Molecular Data SAG will continue advising the AZA community on the integration of DNA data into SSP management, including the use of the new resources developed through the proposed project, beyond the end of the project's formal conclusion.

Citations

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