ORANGUTAN GENETIC DIVERSITY

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In 1985, a project was developed to evaluate the genetic diversity of wild orangutans and to address the questions arising from the Orangutan Species Survival Plan concerns on sub-species hybridization. Additionally, it was hoped that sampling from specific geographic locations would help pull together information on the physical differences observed within and between Sumatran (*Pongo pygmaeus abelii*) and Bornean orangutans (*Pongo pygmaeus pygmaeus*) and the data from, genetic studies of captive populations (Seuanez 1982; Dugoujon et al. 1984; Janczewski et al. in press).

Before field work began in 1988, three years of groundwork were required to develop appropriate technologies, identify study sites and collaborators, obtain government approvals, and acquire adequate funding. Actual sample collection took place in the spring of 1988 on Sumatra, and the spring and fall of 1990 on Borneo. Laboratory analysis of the samples is currently being completed at the National Cancer Institute.

Criteria for the selection of sample collection sites included reports of high orangutan densities, accessibility, available field assistance, logistical support, possibility of government approval, and geographical significance. Once areas were identified collaborative agreements were established with field scientists and/or government agencies and staff to ensure acceptance of our work in each area. Funding sources were also explored at this early stage. In Indonesia, the official counterparts for the project were the Primate Research Center of the Institut Pertanian Bogor and also the Indonesian Zoological Parks Association (IZPA). For the work in Sabah and Sarawak, the Wildlife Department and Wildlife Division of the Forestry Department respectively, were the counterpart agencies. As part of our agreement with the IZPA, we agreed to visit the member institutions and sample their orangutans for karyotyping. This resulted in sampling 50 individuals at six institutions.

Development of new technologies was required to safely obtain samples from wild orangutans and preserve them for later analysis. A biopsy dart (Karesh et al. 1987; Frazier-Taylor et al. 1990) was developed for the purpose of this project, and was used to obtain 3mm diameter X 5 mm deep skin biopsies from free ranging and captive orangutans. The dart was developed and extensively tested using captive animals before wild individuals were sampled. Follow-up

examination of free-ranging individuals showed that the biopsy sites healed quickly and with no complications even in tropical environments.

The biopsy samples were removed from the dart and immediately placed in a 10 ml vial of transport solution (See materials reference). Within a few hours following collection, samples were cleaned by removing hair and foreign material and repeatedly rinsing in fresh transport solution. They were then placed in 2 ml cryotubes containing a freeze media (See materials reference). Thirty to sixty minutes later, tubes were placed in the vapor phase of liquid nitrogen for freezing. Once frozen, the vials were stored in a liquid nitrogen dry shipper or ultra-cold freezer until thawed for tissue culture. DNA harvested from cells grown in tissue culture was analyzed using mitochondrial DNA restriction site analysis, major histocompatibility restriction site analysis, and displacement loop gene sequencing.

Over one hundred free-ranging and captive orangutans in Indonesia and Malaysia have been sampled to date. Survival and successful growth in tissue culture has improved dramatically over the course of the project. Of the 65 samples collected in 1988, only 22 survived. Of the 50 samples collected in 1990, 49 survived. We attribute the higher success rate to more quickly cleaning and freezing the samples after collection, making up fresh sterile transport media every few days, keeping the freezing media frozen ready for use, and improved tissue culture techniques.

Free-ranging animals were samples in five geographically isolated areas: northern Sumatra, East and West Kalimantan, Sabah and Sarawak. Additionally, confiscated animals being held or rehabilitated and released were sampled in these same regions. Analysis of zoo specimens will be handled separately from free ranging populations and individuals with exact capture location documentation.

RESULTS

Even before the completion of the final genetic evaluations, the project has generated positive results in a variety of ways. Information gathered in Indonesian zoos represented the first time that orangutan inventory data was compiled, with their direct input, and distributed among the zoos. This information has formed the basis of an orangutan studbook. The information was also provided to the international orangutan studbook keeper for updating those records.

This study is an excellent example of the benefits of developing a zoo-based, conservation oriented project. The development of the dart and

freezing technology was significantly enhanced by utilizing zoo collections. It allowed access to a wide range of species which could be closely monitored after the darting procedure as well as the opportunity to gather tissues opportunistically for testing freezing techniques. The zoo base also allowed for funding sources to be utilized that would typically not be available for a free-ranging wildlife study, in this case the Institute of Museum Services provided the majority of the funding for the project, with Zoo Atlanta underwriting the costs of the initial work in Sumatra and the Chicago Zoological Society and the Puget Sound Chapter of the American Association of Zoo Keepers providing funds for equipment.

Another result of the project has been the training provided to zoo staff and wildlife managers in Indonesia and Malaysia. In all locations, we were teamed up with counterparts to be trained in the techniques that we were using. This hands-on experience is a rare opportunity for wildlife workers in much of the world. Spending extended periods of time with individuals also provided a chance for long discussions related to captive and free-ranging animal health and management issues. We were frequently used as consultants for health related issues wherever we worked, such as undertaking a simple primate disease survey at a national park at the request of one wildlife department. The team approach provides opportunities to establish long-term relationships that are mutually beneficial. Since working in the field together, we have continued to share information and advice on zoo and wildlife issues and discuss future collaborative projects. As part of our agreement with the Indonesian Zoological Parks Association, we donated a liquid nitrogen dewar, Simmons rifle and Cap-Chur pistol for use by their member institutions, and darting equipment was also donated to the Sabah Wildlife Department.

A spin-off of the project has been the wide-ranging applications of the biopsy dart and freezing techniques. When we started the project in 1985, conventional wisdom said that even if we could get the samples, it was not possible to freeze whole biopsies and have them survive for tissue culture. The development of these two techniques has opened up a whole range of possibilities for genetic field studies and the approach has since been applied to a wide range of species and field settings including big horn sheep, American bison, African hunting dogs, African bush and forest elephants, wildebeest, zebra and okapi.

Cell cultures established from the orangutans have been made available to Indonesian and Malaysian counterparts. One set of samples has been sent to the Primate Center laboratory in Bogor and another set for Indonesian researchers studying familial relatedness of individuals at the Ketambe Research Station in Sumatra.

The genetic analysis of the samples collected should be concluded in the fall of 1994. Displacement loop gene sequencing and analysis of the major histocompatibility complex is being conducted to confirm the findings to date.

Karyotyping of individuals showed:

1. All free-ranging animals as well as rehabilitation candidates were in the correct location for their subspecies.
2. Karyotyping of captive individuals confirmed the suspected subspecific designation in all but one individual. This information is crucial to proper management of captive breeding programs and is essential for studbook development.

The molecular genetics work completed to date has revealed:

1. The Bornean and Sumatran lines have been genetically isolated for roughly 1.5 million years, even though land connected the two islands as recently as 20,000 years ago.
2. Bornean and Sumatrans are genetically at least as different from each other as chimpanzees and bonobos, and more different than other species. Added to reproductive isolation, and morphological differences, it would be easy to justify them being reclassified as two species.
3. Two matriarchal lines exist sympatrically in Sumatra, dating back 600,000 years. This was most likely due to either a bottleneck occurrence or a second immigration from the mainland prior to their disappearance. Also, there is more genetic diversity in orangutans or even Sumatran orangutans than seen in most mammals, including humans.
4. The different populations sampled on Borneo have been isolated for roughly 200,000 years. They are not different enough to warrant sub-species classification. They essentially represent the makings of future sub-speciation. Each population is different but if we look at one individual, you cannot say with 100% reliability where it may have come from. This will resolve some of the confiscation problems because it tells the governments that if it is possible, animals from Borneo should be returned to the region where they came from. But if it is not possible, no significant genetic harm is being done. It also supports new efforts not to release animals into areas with existing populations and instead use the confiscated animals to establish new populations to help flag more areas for protection. This will also help to deal with the disease issues that plague the decision making process for confiscated animals. Because diagnostic testing for some diseases can not be relied upon to ensure the safety of releasing animals back

into existing populations, it would be much safer to release these animals into areas of good habitat where no current populations exist. The results of this study indicate no genetic reasons against establishing new and possibly mixed populations (mixing within a subspecies, not between subspecies).

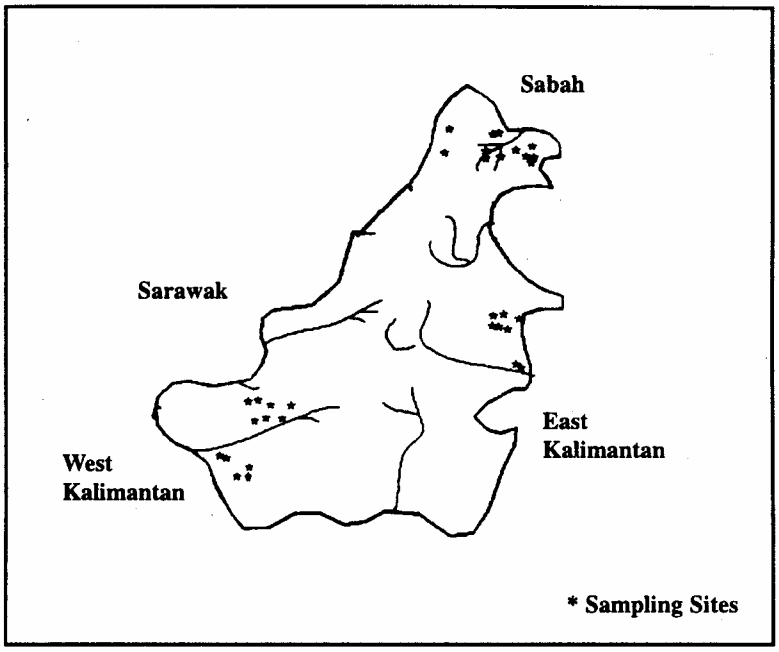

Figure 1. Orangutan genetic sampling sites in Kalimantan and Malaysia.

5. No single population on Borneo shows a lack of diversity that would indicate a need for introduction of new genetic material, though some areas do appear to have more diversity than others.
6. It had been suggested that orangutans in Southwest Borneo are closer to the Sumatran subspecies than they are to the Bornean population. To date the genetic results indicate that this is not the case.

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MATERIALS

Transport Solution: Phosphate buffered (PBS) or normal saline containing Penicillin G sodium, 100 units/ml, Streptomycin sulfate 100 mcg/ml, and fungizone (amphotericin B) 0.25 mcg/ml.

Freeze media: Minimum Essential Media with 10% fetal calf serum (MEM 10%) and 10% Dimethylsulfoxide (DMSO) and containing Penicillin G sodium, 100 units/ml MEM 10%, Streptomycin sulfate 100 mcg/ml MEM 10%, and fungizone (amphotericin B 0.25 mcg/ml MEM 10%.