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PREFACE

Prefaces to this form of publication are frequently staid in format and tone. However, since this group is anything but formal in body or tone, I believe that such rigidity here would be inappropriate.

It is however a'propos, to generously thank those who have made significant contributions toward this project's fruition.

To the editors of this compendium, Sean McKeown and Fred Caporaso, my sincere appreciation for the difficult, thankless job of organizing, checking, crystalizing and generally upgrading the melange' of papers presented.

Special recognition to Herpetofauna International and Hank Molt, and Herpetofauna, Inc. and Tom Crutchfield, Bill Love and Bob Harding, and all the other sponsors, without whose financial support and enthusiasm these proceedings might not be possible without significantly raising the symposium registration.

This publication would not have been possible without the additional efforts of Dr. Richard Ross, Program Chairman, David Barker, Co-ordinator, and Mike Goode, Curator of Reptiles of the Columbus Zoo and Host Chairman for the 8th International Herpetological Symposium.

To these people and all the others, the host committee, the participants, the proceedings recipients...who support and boost the IHS goals and results, this volume is dedicated with my gratitude and appreciation.

To the office staff of the Catoctin Mountain Zoological Park and Zoological Consortium, Inc. and especially Mary Anne Hahn who has volunteered hundreds of hours annually for the past eight years to insure that the typing, editing, organizing, registration, orders and general public services are done in a caring and effective way, my deepest sympathies.

Finally, the contributions all the above duly acknowledged, I request that any and all errors or criticism, constructive or otherwise be attributed and directed to me.

Richard A. Hahn
Symposium Series Co-ordinator

TABLE OF CONTENTS

SERPENTES

HERPS AND FEDERAL WILDLIFE PERMITS

Steve Funderburk

INTRODUCTION

The United States has implemented several laws to protect and conserve wildlife and plants. One of these laws, the Endangered Species Act (ESA), imposes certain restrictions against collection and trade in protected species, including 99 species of reptiles and 16 species of amphibians. The ESA is also the law that implements U.S. participation in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), a treaty shared by 86 Party Nations to prevent over-exploitation of wild animals and plants due to trade. Over 250 species of reptiles and 10 species of amphibians are protected by CITES. This paper discusses general wildlife trade restrictions and prominent features of the ESA and CITES as they affect amateur and professional herp breeders and enthusiasts.

For over a decade many species of wild animals and plants have been protected against collection and trade by the Endangered Species Act (ESA or ACT). Since 1977 the United States has restricted import and export of species protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Although trade restrictions imposed by the ESA and CITES are instrumental in preventing over exploitation of rare and heavily traded species, they can pose difficult permit problems for persons trading in protected species, whether such trade is for commercial profit, research or captive breeding. Problems usually arise because persons are ignorant of general trade restrictions and permit requirements. The information given in this paper should clarify Federal wildlife permit requirements for anyone actively involved in trading herps, for whatever reason. The paper also discusses the historical background of the ESA and CITES.

General Background:

Efforts to protect endangered species in the U.S. began in 1966 when Congress passed the Endangered Species Preservation Act. This law did not prohibit anything but did authorize funds for native species management and research. In 1969 the Act was amended and renamed the Endangered Species Conservation Act. The 1969 Act included an import restriction on foreign endangered species and stimulated the concept of "recovery plans" that were to outline methods to promote the survival of wild endangered species. The biggest change occurred in 1973 with passage of the present Endangered Species Act (ESA), which replaced earlier laws. The 1973 Act imposes restrictions on the take or collection of endangered species found in the U.S. and prohibits import and export of all listed species. The 1973 Act also prohibits interstate and foreign commerce in the course of a commercial activity.

The ESA broadened the scope of protection by enabling any vertebrate, invertebrate, or plant to be listed and by enabling subspecies or depressed population of certain species or subspecies to be listed. Many herps are listed at the subspecies level. Examples include the Eastern indigo snake Drymarchon corais couperi and Santa Cruz long-toed salamander Ambystoma macrodactylum croceum. At present 785 species are protected by the ESA, of which 99 are reptiles and 16 are amphibians. At least 70% of the listed herps are foreign species; only 34 species are native to the U.S.

A new category of species known as "threatened species" was introduced by the 1973 Act. Whereas endangered species are those plants and animals facing extinction, threatened species are not presently facing extinction but may become endangered in the foreseeable future. Special rules can be issued for threatned species (but not endangered species) to allow for certain activities that are generaly prohibited. Special rules exist for the American alligator Alligator mississippensis, green turtle Chelonia mydas, olive ridley turtle Lepidochelys olivacea, loggerhead turtle Caretta caretta, and San Marcos salamander Eurycea nana.

CITES, also known as the Convention, Endangered Species Convention, or Washington Convention, was formulated in 1973 when an international meeting was held by 88 nations in Washington, D.C.. CITES entered into force July 1, 1975, after it was ratified by 10 Party Nations. Section 9 of the ESA implements the Convention in the U.S.. Although the U.S. ratified CITES in 1974, it did not enforce it until May 23, 1977, when specific Federal regulations went into effect (Title 50, Code of Federal Regulations, Part 23). The Convention is designed to regulate trade in wild plants and animals so that such species are not over-exploited. To date CITES has been implemented by 86 nations.

Species protected by CITES are subject to import and export restrictions and are listed in three appendices to the treaty. Appendix I includes species threatened with extinction that are or may be adversely affected by trade. Appendix I species may not be traded for primarily commercial purposes. Many crocodiles are included in Appendix I. Appendix I includes species that although not now necessarily threatened with extinction may become so unless trade in them is strictly controlled. This appendix also lists species that must be subject to regulation in order that trade in other currently or potentially threatened species may be brought under effective control. Such listings, known as look-alike listings, are required because of the difficulty in distinguishing at the port of entry or exit the rare species from common but similar looking species. All crocodiles and boas not listed in Appendix I are included in Appendix II. Appendix II includes species that any Party Nation identifies as being subject to regulation within its jurisdiction for purposes of preventing or restricting exploitation, and for which it needs cooperation of other Party Nations in controlling trade. All together, the three appendices include about 2,400 species of animals and 30,000 species of plants. Of these, over 250 species are reptiles and 10 species are amphibians.

Many species are protected both by the ESA and CITES. Of the 99 species protected by the ESA, 80 are protected by CITES and almost all of those are listed in Appendix I. Three of the ten amphibians listed by the ESA are also listed in Appendix I. Several major groups of reptiles are highly protected by both the ESA and CITES; they include the crocodiles (Crocodylia), marine turtles (Chelonidae), Madagascar boas (Acrantophis spp.), Round Island boas (Casarea and Bolyerea), and ground iguanas (Cyclura spp.).

As you can see, both the ESA and CITES restrict international trade. That is the main feature they share. The ESA differs from CITES in that it is strictly a U.S. law, not a treaty. CITES concentrates on regulating international trade in wild fauna and flora but has no direct restriction against species harvest or collection like the ESA does. Unlike CITES, the ESA allocates money to recover species through captive breeding, research, and habitat preservation. The ESA and CITES are clearly separate from each other; permit problems often occur when the ESA and CITES are regarded as the same or when they protect the same species.

ESA and CITES RESTRICTIONS

The ESA and CITES protect listed species by prohibiting cetain activities that are known to be detrimental to wild plants and animals. The prohibited activities are summarized below:

ESA
* Take (kill, collect, harm, harass)
* Import or Export
* Interstate or foreign commerce in the course of a commercial activity
* Sale, or offer for sale without a warning that Federeral permits are necessary before sale is consumated.
* Possession of illegally acquired species.

CITES
* Import and export of Appendix I species.
* Export of Appendix II and III species.
* Reexport of any CITES-listed species.
* Possession of smuggled CITES-listed species.

The ESA does not distinguish between export and re-export as CITES does. The prohibited activities affecting herp breeders and enthusiasts are primarily interstate and foreign trade.

Permit Requirements

Permits may be obtained to authorize any of the prohibited activities mentioned above provided certain conditions are met. Tables 1 and 2 summarize ESA and CITES general permit requirements. Persons who would like to export CITES-listed herps that they have bred in captivity may apply for a Letter of Authorization (LOA). The LOA is basically a pre-clearance for any number of CITES export permits that persons needs to ship qualified herps to foreigners.

Permit Exceptions

Several permit exceptions under the ESA and CITES apply to live reptiles and amphibians that eliminate the need for the standard permits mentioned above. Following is a brief description of each exception for live herps.

Endangered Species Act

Pre-ESA Specimens: This is basically a "grandfather clause" for specimens held prior to the date the species was listed under the ESA. The earliest pre-Act date is December 28, 1973, for all species listed prior to the 1973 Act. The date of listing in the Federal Register applies to all other species listed after December 28, 1973. The pre-Act exemption does not apply to interstate or foreign commerce transactions but does permit non-commercial import and export and interstate movement without a permit. Pre-Act status must be documented in a form described by regulation (50 CFR 17.4).
Special Rules: Some Threatened Species Special Rules eliminate standard permit requirements. For example, take of American alligators in Louisiana and San Marcos salamanders in Texas are governed only by respective State Regulations.
Loans and Gifts: Endangered or threatened species may be loaned or given to anyone else in the U.S. provided it is not loaned or given for some type of financial gain or profit. Barter cannot occur without a permit.
Intrastate Commerce: ESA restrictions do not apply to the buying and selling

of legally acquired endangered and threatened species solely within the boundaries of a persons State of residence.

Captive-bred Wildlife: The Captive-bred Wildlife (CBW) permit is a special type of ESA permit. It is a system whereby one can become registered with the Fish and Wildlife Service to trade in endangered and threatened wildlife that are not native to the U.S., that are captive-bred in the U.S., and that are to be bought or sold for enhancement of propagation or survival. The CBW permit facilitates trade by eliminating the need for individual permits for each commercial transaction. A "registered" person can buy and sell U.S. born endangered or threatened wildlife in interstate commerce with other registered persons. However, persons trading between themselves must be registered for that category of animal. A person registered for endangered pheasants cannot buy endangered boas from someone in a different State under the CBW permit; a standard ESA permit would be necessary. A person may become registered for as many species or families as they have husbandry and breeding experience in and adequate facilities for. Properly marked animals may be exported for breeding purposes if foreign recipients are shown to be qualified to care for and house animals. No wildlife may be imported under a CBW permit if they had not been exported under a CBW permit. Foreign born endangered or threatened species may be imported under standard ESA permits.

CITES

Pre-CITES Specimens: CITES-listed species acquired prior to the date CITES listed the species are exempt from import, export and re-export restrictions. However, to enjoy this exemption, a Certificate of Pre-Convention Wildlife must be acquired before import, export or re-export. A U.S. Pre-Convention certificate is not necessary if a valid foreign Certificate for Pre-Convention Wildlife has already been issued.

Captive-bred Specimens: CITES-listed species that are reliably bred in captivity to the second generation may be exempted from CITES restrictions. A CITES Certificate of Captive-bred Wildlife is necessary and may be acquired upon satisfaction of established CITES captive-bred criteria. Qualifying Appendix I specimens that are to be traded for primarily commercial purposes are treated as Appendix I specimens and original permits are issued for each export instead of a certificate.

Permit Procedures

ESA and CITES permit/certificate applications may be obtained from the Federal Wildlife Permit Office (FWPO), P.O. Box 3654, Arlington, Virginia 22203 (telephone number 703-235-1903). The FWPO Office has developed special easy-to-understand forms for each type of permit/certificate. If a species to be imported or exported is covered by both the ESA and CITES, then ask only for an ESA permit application. Applications must be submitted with a $25 fee to defray processing costs; however, governments, public institutions and the like are exempt from fees.

Applications are evaluated as quickly as possible. Often they have to be reviewed by several offices besides the FWPO and it is important that as much detailed information as possible be provided. Expect at least a 30 day review period for CITES and CBW permits and 75 days for standard ESA permits.

Shipping Requirements

Any shipment of wildlife imported or exported must enter or exist through a wildlife designated port (New York, Chicago, Miami, New Orleans, Dallas/ Ft. Worth, Seattle, Los Angeles, San Francisco, and Honolulu) and be declared to a Fish and Wildlife Service Wildlife Inspector for clearance and inspection. Additionally, a Wildlife Declaration (Form #3-177) must be filled out upon import or export for each shipment. This form is available from the Wildlife Inspector and may be completed at the time of shipment. Any permit needed to authorize import or export must be presented to Wildlife Inspectors and validated. CITES export or re-export permits must accompany the shipment. Mailing herps internationally is subject to all permit and declaration requirements. Before mailing a shipment, it must be declared to a Wildlife Inspector for clearance and inspection. Associated permits must be validated and enclosed with the mail package containing herps.

CONCLUSION

Many species of herps are protected by the ESA and/or CITES. Lists of protected species are available from the FWPO (address above). Permits are needed for import, export and re-export of ESA and CITES-listed species; interstate and foreign commerce of ESA-listed species, and take of ESA-listed species within the U.S.. Permits can be issued only for certain purposes. Several permit exceptions may apply. General Federal wildlife shipping requirements must be met. Keep in mind that the Lacey Act requires persons to obey all local, State, Federal, and foreign laws. Questions about Federal Wildlife Permits should be addressed to the FWPO.

Table 1. PERMIT REQUIREMENTS AND PROVISIONS UNDER THE ENDANGERED SPECIES ACT.

Species Status	Permit Needed to Authorize	Permit May Be Issued For
Endangered	* Import or Export * Interstate/foreign commerce * Take from the wild	*Scientific Research *Enhancement of propagation or survival
Threatened	* Import or Export * Interstate/foreign commerce * Take from the wild	* Scientific research * Enhancement of propagation or survival * Zoological exhibition * Educational display * Special purpose

Table 2. PERMIT REQUIREMENTS AND PROVISIONS UNDER CITES.

Species Status	Permit is Needed To Authorize	Permits May Be Issued For
Appendix I	* Import, Export or Re-export (1)	*Exceptional activities that are not detrimental and are not for primarily commercial purposes
Appendix II	*Export or Reexport	* Any activity not detrimental to the species
Appendix III	*Export or Re-export	* Any activity (3)

(1) Import of Appendix I species requires, upon import, presentation of export/re-export permit from exporting/re-exporting nation: issuance of dix I export pemit may not occur until importing nation issues CITES permit.
(2) No U.S. CITES permit is required to import Appendix II or III wildl the specimens must be accompanied, upon import, by a CITES export or re- permit, or Certificate of Origin for Appendix III specimens originating country that did not list the species under CITES.
(3) Appendix III wildlife must be exported from listing country with permit; non-listing countries issue Certificates of Origin for export.

Federal Wildlife Permit Office, Arlington, Virginia

HOW IT PROTECTS WILDLIFE

Terry Lilley

The material in this article is actually nothing new or revolutionary; in fact,the contents were written over 200 years ago. We all learned the principles set forth in this article when we were in grade school, but it is apparent that most of us have forgotten them. This article was written to once again remind you how great America really is and that it is up to its people, under the free enterprise system, to protect our reptile wildlife. We must set our country back on track and give the freedom and the control of wildlife back to the people.

The United States of America was built on capitalism and free enterprise which made this country great. My father laid his life on the line in World War II to protect our way of life and our way of doing business, and I am committed to laying my life on the line to protect it here from nothing more than ourselves. The people in this country are not owed anything from our government. Remember, "We are the government." It is our duty as American citizens to protect free enterprise, and we cannot expect our government to do it for us. Wildlife can only be fully protected by using free enterprise principles. A Frenchman, in the 1960's, came to American on behalf of his government, with an assignment to figure out what makes America so great. He later published a book; in it, he said, "America is not great because of its government, its churches, its schools, but America is great because of its people, due to their drive to be free."

Old Method of Wildlife Protection;

In the past twenty years, wildlife has been protected by an attempt to keep it out of the hands of the people. This has been done by our government putting reptiles on the protected list and basically saying that the public either cannot protect it or does not know how. For the past twenty years, this system in America may have been the only known system to attempt a protection program. This system causes people who love and wish to work with wildlife to become criminals; it causes courts to have to work on cases; it takes jobs away from the people, and I am not convinced that it protects wildlife at all, because it causes a flourishing black market and poor care of the reptile being smuggled.

I believe that no one has the right to criticize anyone who is at least trying to find an answer to a problem, until he finds a better answer himself. In the past five years, an obviously better answer to wildlife protection has been developed; it is called Commercial Captive Breeding.

New Method of Wildlife Protection

In this country, we operate a system of doing business called Free Enterprise. That means you can get paid money for the effort you put into a business. For more effort, you can get paid more. It also allows you to fail, if you do not put forth any effort. Free Enterprise creates pride and motivation that creates new products and new markets. This causes more jobs for the people and a better life style. When we make money for our effort, we put the money back into our business, in order to allow us to grow and produce more and new products. This creates competition among businesses and keeps up the quality of the product produced. For the most part, our government should stay out of our business and let business itself, through competition, develop moral and ethical standards. These principles are set forth and protected in our constitution, and I, personally, believe in them.

When a dealer collects a reptile and sells it to a pet shop, he generates an income. This money goes back into collecting more reptiles and eventually will lead to over collecting of many reptile species. This also leads to government agencies applying large amounts of tax dollars to try and stop this type of collecting. It creates problems for anyone who truly wants to protect reptiles and stay within the principles set forth in the constitution.

In recent years, many herpetologists and zoos have become very successful at captive breeding of reptiles. These reptiles, when sold, produce an income related to the amount of effort the breder put into his work. This income normally goes back into breeding more and new reptiles, which creates new markets and jobs that once did not exist. These reptiles which are now available compete in price with imported reptiles, thus creating competition. This competition drives the quality and quantity up and the prices down. The imported reptiles usually cannot compete, and the captive-bred reptiles become the entire market. Once this happens, it is no longer profitable to collect reptiles from the wild in large quantities for resale. This, in effect, has totally protected the wild populations from collecting (other than small numbers for new blood lines), and the money generated has gone for a good purpose. Also, by establishing this type of free enterprise protection, our government does not have to spend millions of dollars to try and stop people from collecting wildlife. It creates jobs, develops research and education, and creates finances, so that we can better our life styles. This type of wildlife protection makes heroes out of commercial breeders instead of criminals.

Examples of This Type of Protection

The Eastern indigo snake Drymarchon corais, is a protected species in the USA; you cannot sell captive produced specimens across state lines without a permit, which is very difficult to get. This causes people to smuggle wild caught indigos and collect them in Florida. Since captive breeders cannot create an interstate market, they cannot compete with the black market prices and the smugglers' businesses flourish.

If captive breeders could sell any captive produced indigo anywhere they wanted, then new markets would open. Many people would breed the indigo because the price for the captive-born young is high enough to make some good money. Once several thousand captive-born indigos are available, then competition with the smuggled indigos will begin. The breeder can finance new methods of breeding in quantity, to lower his price, which will soon cut the collector out of the market. Within two to five years, Eastern indigos can be produced in such quantities that it would not be worth your time to collect wild ones. Captive-produced animals would be much healthier, prettier, and tamer than wild caught. The money brought in to the breeder would go toward breeding more snakes and not collecting more (except for new blood lines).

This concept has worked for many species already. It is easier and cheaper to buy captive-born Blairs kingsnakes, Sinaloan milk snakes, leopard geckos, horned frogs, and Burmese pythons, that it is to collect wild ones. The government can also save large amounts of tax dollars, because business is now protecting these snakes instead of the government.

I think free enterprise, in less than twenty years can fully protect wildlife at a much greater rate than total government protection could do in the next 200 years. It is up to us to change laws and open this country back up to a type of business that is supported and guaranteed under our constitution. We all are looking for one answer to our wildlife protection problem, and we all should work together. Captive breeders are not criminals and should be supported. What we are doing will lead to the best possible chance for man to live in harmony with nature on our small crowded planet. Free enterprise made this country great; why not continue with a proven method of wildlife protection, one that supports not only reptiles but the American system?

Central Coast Reptile Research Center, Morro Bay, California

THE VALUE AND APPLICATION OF FROZEN TISSUE COLLECTIONS IN THE PROPAGATION, MANAGEMENT AND CONSERVATION OF HERPETOFAUNA

Peter A. Rosenbaum, Richard C. Atkinson

Mark S. Hafner, Herbert C. Dessauer

INTRODUCTION

The main purpose of this presentation is to publicize advances made in the field of ultracold storage of tissues (cryopreservation), and how this technology can enhance your present breeding program(s) and benefit science and society. By reviewing some application of this and related biomedical technologies, we hope to interest you in getting more involved in research employing these techniques for the mutual benefit of yourself, your breeding groups, science and society in general.

Much of the material presented here stems from a report to the National Science Foundation made by a panel including numerous collection managers, who met in May, 1983 at the Academy of Natural Sciences of Philadelphia, sponsored by the Association of systematic Collections (Dessauer and Hafner, 1984).

Of the six major recommendations of the workshop panel, two are especially relevant to this form, including:

1) "A limited number of existing frozen tissue collections in the U.S. should be designated as FROZEN TISSUE DEPOSITORIES, charged with accepting, preserving and distributing tissues..".

2) "Inventories of collection holdings and advances in the fields of tissue cryopreservation and tissue collection curation should be publicized for the benefit of all."

The subject of FROZEN TISSUE DEPOSITORIES will be discussed later in this paper. The publicizing of the advances in the field of tissue cryopreservation and their current and potential future applications in the propagation and conservation of herpetofauna (especially rare, threatened, or endangered species) are the purposes of this presentation.

Rational:

With accelerated destruction of the earth's wildland, wildlife and genetic carrying capacity of wildlife populations are diminishing, and gene pools are declining and fragmenting (Foose, 1983). Preserving diversity on this planet, both in numbers of individuals and diversity of species has become a global issue. This international concern has bound various individuals from various disciplines to a common goal. For example, the International Union for the Conservation of Nature and Natural Resources (IUCN, 1980) is devoted to the propagation of biotic diversity on this planet. Similarly, the American Association of Zoological Parks and Aquariums (AAZPA) has adopted a collective strategy to address this problem by its studbook registry (International Species Inventory System or ISIS) and has plans to manage selected species through Species Survival Plans (SSP). Some reptile species are currently managed by Species Survival Plans, but as yet, ISIS does not include reptiles and amphibians (See Slavens 1982) inventory of captive holdings or reptiles and amphibians.

One strategy to preserve rare, exotic , threatened or endangered species uses cryopreserved tissues, especially gametes (sperm and eggs) and embryos. Cryopreserved genetic material has been successfully applied in captive breeding programs of many wild and domesticated species of birds and mammals (e.g. Gee, 1983; Gee and Sexton, 1979; Gee and Temple, 1978; Seager et al., 1978; Waladsen et al., 1977). To date, cryopreserved materials are vastly under used in captive reptile breeding programs. While some methodological problems associated with the cryopreservation of herp tissues and gametes have been overcome (e.g., Brazaitis and Watanabe, 1982; Gorzula et al., 1976), many technical problems remain to be solved before the technology can be applied to captive reptile breeding programs.

Technological breakthroughs and development of new biomedical techniques are now commonplace. Hardly a day goes by when the media are not reporting some new development or novel application. Last year at this meeting, we learned how ultrasonography was being successfully applied to determine litter size and viability in certain snakes (Tolson et al., 1984). Just a few weeks ago the first human infant was born after having been stored as an embryo at ultracold temperatures and subsequently implanted into its mother's womb. Similarly , last month the death of South American millionaires brought up questions of what to do with the frozen embryos the couple left behind in Austrailia. Tissues from some animals dead for many years have been found to retain biologic activity useful in molecular studies. Even some animals, extinct for thousands of years, but frozen in nature, have been found to retain significant biological activity.

Basic studies of the uses of cryopreserved genetic material in the propagation of reptiles is largely our responsibility. We are pioneers in breeding the rapidly vanishing forms of herpetofauna. We are devoted to the ultimate survival and conservation of these species. It is up to us to solve the practical problems, develop innovative techniques and, of course, judiciously apply existing technologies to the species we are working with. No one has greater interest in herp preservation, and no one is likely to solve our problems but us. Experience with birds, mammals, and other animals suggests that solutions found to be applicable to one species or taxon may not be useful with others.

Before any of this essential research can take place, raw materials must be available for study. Especially for endangered species, these materials are, at best, difficult to acquire and accumulate. Frozen tissue collections represent a primary resource for maintaining viable tissues necessary for breeding programs, molecular studies in evolutionary biology, and studies in systematics, genetics, biochemistry and immunology. If cryopreserved tissues had not been available, many important studies in the past could not have been undertaken (see Smith et al., 1982). This same principle holds for applications to captive breeding programs and conservation efforts.

Frozen tissues have been invaluable in many areas of research on vertebrates (see Smith et al., 1982 for a general bibliography; and Russell, 1960; Dessauer, 1970; Mao And Dessauer, 1971; Jimmez-Porras, 1961 and 1964 for some reptilian examples). A brief description of three studies employing cryogenically stored reptilian tissues will illustrate both the realized and potential value of cryopreserved reptilian tissues in basic and applied research, including captive breeding programs.

For many years, the American Museum of Natural History has maintained and bred kingsnakes, Lampropeltis getulus. Part of their research protocol requires that tissues from members of this breeding colony be cryopreserved. The availability of these tissues has enabled detailed pedigrees of 92 individuals to be constructed (Dessauer and Zweifel, 1981). Information about the genetic relationships among members of a breeding colony is always important if the colony is to remain vital over many generations and is especially important to endangered species breeding programs.

In another case, a single female kingsnake, L. getulus, mated on successive days to two different male snakes, which resulted in six offspring. By collecting and cryopreserving tissues from all potential fathers, the mother and the young, it was possible to document, biochemically, that each of the two male snakes had sired three young each (Zweifel and Dessauer, 1983). Documenting multiple insemination and determining correct parentage are important considerations for successful breeding programs. This is especially true over many generations or where the populations originated from a limited founder population or where subspecific affinities between mating individuals are uncertain.

Although phylogenetic relationships are of more interest to systematists than to breeders, a study on the systematics of the order Crocodilia is noteworthy because of the active participation many of you took in collection of these samples. Recently, one of our biochemistry students collected blood samples from all of living crocodilian species in order to study the systematics of that group, using an immunological and biochemical approach (Densmore, 1981). Many of you permitted your often irreplaceable specimens to be subjected to venipuncture so that raw materials for this study could be obtained. Much valuable information was learned from this work, including a recent report (Densmore and Dessauer, 1984) describing close biochemical affinities between the true gavials (Gavialis) and the false gavials (Tomistoma). This study has also provided biochemical evidence suggesting a closer phylogenetic relationship among living crocodilians (perhaps monophyly) than has been suggested on the basis of other evidence (e.g., paleontology).9

Cryopreserved Collections VS. Formalin Fixed Collections

It is important to emphasize the usefulness of cryopreserved materials as opposed to traditional formalin or alcohol preserved materials. First, cryopreserved materials can be collected over many years and remain viable. This often makes collection of fresh materials for analyses unnecessary. Second, because cryopreserved materials remain viable for long periods of time, they can serve as "banks" of genetic material. This is important for retrospective studies as well as to preserve this genetic material from vanishing life forms before they become extinct. In this sense, extinction need not be forever, if suitable genetic materials are preserved before wild and captive populations disappear. Cryopreservation is also a mechanism for preserving genetic diversity at considerably less expense than is required to maintain either captive or wild populations. Finally, there are already many formalin-fixed materials (skulls, skins, etc.) for most taxa in existing natural history collections. Unfortunately, formalin-fixed materials are of little or no value in molecular or propagation studies. Special efforts are needed to secure tissue samples from threatened and endangered species before human encroachment on habitats eliminates large numbers of species from our planet (Wake et al., 1975; Myers, 1978; Russell, 1978; Genome Conservation, 1980; Ehrlich and Ehrlich, 1981; Frankel and soule, 1981; Dessauer and Hafner, 1984). Animals preserved now will be the raw materials (the data base) for a variety of future biological investigations ranging from breeding studies to behavior, from biochemistry to systematics and DNA sequencing.

Applications of Frozen Tissues to Captive Breeding Programs; Present and Future

From a practical viewpoint, frozen tissue collection technology is already being applied to captive breeding programs and is demonstrating direct beneficial results. More often than now, it is the commercial breeder of domesticates who is the driving force behind direct applications of frozen genetic materials in breeding programs. While the financial rewards for work with reptiles may be less than with, say, domesticated ungulates, there are both idealistic as well as practical rationales for using frozen genetic materials in reptile breeding programs. The tangible, real values in achieving successful reptile breeding programs are obvious. Ultimately, successful applications will result in an increased yield of healthy living animals. Modern biomedical technologies borrowed from various disciplines (e.g., pathology, genetics, ethology, virology, and reproductive physiology) are currently being used in an attempt to confront the many problems associated with endangered species propagation. Employing existing technologies, frozen tissues can and should be used by managers of captive breeding groups in various ways. A few examples will illustrate how a variety of studies have proven to be valuable in ongoing breeding programs and these, in turn, will suggest future applications for properly preserved frozen tissue material in propagation programs.

Parentage

It is not uncommon for the genetic identity of an animal born in captivity to be unknown or uncertain. Often, this is the result of animals having contact, and therefore access to breeding, with more than one potential mate (e.g. group caging). Establishing correct parentage (maternity or paternity) can be very crucial, especially among endangered species for which inaccurate studbook data (ISIS) can have detrimental genetic effects (Benirschke and Kumamoto, 1983; Benirschke, 1983). Inaccurate studbook data can lead to excessive inbreeding, admixture among subspecific forms, subspecific hybridization and/or genetic drift. All of these, in any combination, can reduce the vitality of breeding stock. Recently, biochemical techniques using frozen tissues documented maternity in a python breeding group (two females and one male snake housed together). It was determined that the biological mother was not the snake guarding the clutch of eggs (H. Dessauer, pers. comm.).

Genetic changes over time:

It is common for breeding colonies to stem from a relatively small number of original founders. Under such conditions, it takes but a few generations of poor management for genetic bottlenecks or genetic drift to have detrimental effects on a breeding group. Loss of genetic variability due to founder effects have been documented (Ryder et al., 1981). With stored frozen gametes and embryos, genetic bottlenecks can be overcome by introducing new genes from cryopreserved materials and thus rejuvenate the breeding population's gene pool. This, of course, can also be done by introducing new animals for infusions of new genetic material. However, applications utilizing frozen tissues (e.g. frozen semen for insemination) are much more cost effective and protect wild populations from hunting pressures. Changes in the genetic composition populations from hunting pressures. Changes in the genetic composition of a breeding population can only be documented before alteration (e.g. by storing away frozen gametes and embryos). Then, and only then, can changes over time be assesed and appropriate measures undertaken to reverse them.

Matching breeding with respect to subspecific affinities: While hybrid vigor is often sought in domesticated plants and animals, subspecific hybrids are generally less valuable (and less desirable) than animals bred to retain their subspecific integrity. Subspecific status can usually be resolved by comparison with frozen tissue samples of known origin. Morphological characteristics frequently do not permit accurate classification with respect to subspecific status. Biochemical tissue comparisons are therefore increasingly used to solve problems such as this. For example, management of the Bornean (Pongo pygmaeus pygmaeus) and Sumatran (P.p. abeli) populations of orangutans (Seuanez et al., 1979) depends on the ability to identify accurately these two races. Orangutan subspecies are readily distinguishable by chromosomal (karyotypic) analysis.

It is very important to match exotic reptiles with respect to geographic and genetic similarities prior to breeding. Breeding rare races with common sister races functionally results in the loss of the rare subspecies' genetic uniqueness. (For example, New Orleans recently witnessed a deluge of subspecific hybrids between the Burmese python (P.m. molurus) and the Indian pink-faced python (P.m. bivitatus).

Health related applications: Tissues from sick animals that are cryopreserved can be stored over long periods of time, thus permitting detailed pathological studies. Many viral and bacterial agents can be identified long after their collection. In this way, mysterious epidemics within breeding groups can be investigated and, hopefully, recurrences prevented.

Retrospective studies: Tissues collected at a given point in time can be compared with tissues collected from the same animal or breeding group at another point in time to document changes. The effects of new pathogens in the environment, environmental changes, and gene pool changes can be documented. For example, armadillo sera collected between 1958-1962 in Louisiana were found to be ELIZA-positive for Mycobactium leprae, nearly eight years before the first known case of leprosy in armadillos was reported (Dessauer and Hafner, 1984).

Gene pool preservation

Ultimately, the goal of frozen tissue collections is that they can truly serve as "genetic banks" for preserving the gene pools of species no longer found either in captivity or in the wild. Reconstituting species and the habitats they require is a dream for some, and pure fantasy for others. However, the history of science teaches that the science fiction of one era can often be realized in a later era.

Neither wildlands, refuges, zoological parks, or private collections have the capacity to maintain sufficient genetic diversity for the ever increasing number of threatened species (Conway, 1980). This limited carrying capacity will inevitably lead to loss of genetic diversity due to extinction, genetic drift and inbreeding (Senner, 1980). As the very vitality of a species depends on preservation of genetic variability, its loss is hardly trivial. Once lost, genetic diversity cannot generally be restored.

Cryopreserved gametes and embryos represent a partial solution to this extremely complex problem. An individual whose gametes are cryopresevel can supply the genetic material necessary to produce offspring long after the donor's death.

Currently, ova present the most difficult technical problem as far as cryopreservation is concerned. In most cases, our knowledge of ovum preservation is limited to a few species. On the other hand, spermatozoa and semen from a variety of species, including birds, mammals and some reptiles, have been cryopreserved and have been shown to retain their viability over extended periods of time (Graham et al., 1978; Lake, 1978; Sexton, 1976; Sexton and

Gee, 1978). Embryo storage has fundamental advantages over gamete preservation in that a whole diploid organism (not just haploid cells) is being saved (Maure, 1978; Mazer, 1980, Polge, 1978). Embryos from endangered species have been transferred to non threatened species for implantation and gestation (Benirschke, 1983). By experimenting now with non threatened species, it is not unrealistic to envision the day when embryos from rare and endangered reptiles are successfully propagated using non threatened, related species (see Foose, 1983, and Benirschke, 1983). We need to start with the easiest and work our way up to more difficult problems. For example, one might attempt to breed water snakes with garter snakes or vice versa or among various common iguanids. Mammalian successes have already been achieved (Seidel, 1981; Foose, 1983; Benirschke, 1983). We must begin experimentation with these techniques now if we are to bridge the technological gaps preventing the application of these techniques to reptile breeding programs.

It is known that bird gametes and embryo storage require different techniques from those used for mammals; we will undoubtedly find that reptiles present their own unique problems. However, the promise of enhancing genetic diversity, eliminating deleterious alleles, rejuvenating gene pools, prolonging the existence of life forms by using gametes and embryos even after the death of the donor, conducting out of season matings, and many yet undiscovered applications stemming from cryopreserving genetic material can be a reality. We urge you to consider strongly how your breeding programs might benefit from the use of cryopreserved materials. Make appropriate contacts with institutions willing to accept tissue samples, collect the tissues, and begin getting "your feet wet" working in these critical research areas. Your input is vital to the successful development and application of tissue cryopreserved genetic materials in captive reptile propagation.

The Practical Side: Who, Where, and How

Who and where: Persons interested in making use of frozen tissues in their work and those interested in donating specimens of rare, exotic, threatened or endangered species should make personal contact with the collection manager closest to them. A directory of existing collections and their holdings was compiled by Dessauer and Hafner (1984). It includes the collection's size, location, person in charge, kinds of materials housed in the collection, taxa and geographical regions represented, and the major areas of study undertaken using the materials in the collection. In most instances, collection managers (whose names are given in the directory) are quite willing to help. Also, several larger collections in the U.S. may soon be designated as Frozen Tissue Depositories charged with "accepting, preserving and distributing tissues" (Dessauer and Hafner, 1984). If you encounter difficulties finding an institution to work with, contact us (specifically H.C.D. or M.S.H.) at LSU. We have a keen interest in herps and extensive worldwide holdings of tissues from reptiles and amphibians. With our international contacts, we will do our best to help you directly or to make the appropriate contact with the institution most suitable to your needs and interests.

How: Collecting and preserving frozen tissues (including gametes and embryos) can be carried out, even in the field, by persons with minimal training. While procedures vary depending on the organism and type of tissue being preserved, most tissues share many of the same requirements (see Wake et al., 1975; Ahlquist, 1981; Smith et al., 1982; and Dessauer and Hafner, 1984).

Tissue Collection: Animal tissues are best sampled while the animal is still alive. Tissue samples should be taken from recently expired animals as soon as possible or the entire animal frozen immediately. Many tissues, including blood, skin and muscle tissue biopsies, and gametes (especially sperm) can be samples from living animals without serious ill effects on the specimens. In situations where an animal is obviously dying, it is often desirable to euthanize the animal to avoid the ill effects of decomposition after death. This is often a difficult judgement call for the breeder to make between the possibility of an animal's surviving vs. its value as a properly prepared specimen. Animal tissues can be packaged with commercially available materials (i.e. "cryotubes", "cryobags", and "cryosleeves"). However, tightly wrapped aluminum foil, excluding as much air as possible, is a suitable interim substitute. Details of the methods for preserving tissues can be found in Dessauer and Hafner's (1984) report.

As soon as possible after collection, tissues should be quick-frozen by dropping the cryotube directly into liquid nitrogen or by covering cryotubes with dry ice. For interim storage, household freezers are adequate. From a practical standpoint, most specimens can be placed in a plastic bag, wrapped in aluminum foil, and kept in a household freezer for short-term storage (-40 degrees C) until delivery (or pick up) can be made to the tissue collection for long-term storage.

Tissue transport: Importation and transport of frozen animal tissues are generally subject to the same regulations imposed on standard museum specimens (e.g. skins, skulls, and fluid preserved specimens). The Center for Biosystematics Resources of the Association of Systematic Collections (Museum of Natural History, University of Kansas, Lawrence, Kansas 66045) will provide interested persons with current information on U.S. Federal Wildlife Regulations, federally controlled species, and state protected species. Also, commercial mailers are available.

Tissues transported by air are generally shipped on dry ice or in liquid nitrogen, both of which have specific federal regulations governing their transport (see "Restricted Articles" under the International Air Transport Association Dangerous Goods Regulations, 24th ed. December, 1982).

You will not be surprised to learn that the federal regulations pertaining to the transport of frozen tissues (especially bird tissues) are complex, confusing, and frequently result in delays in obtaining permits. While one can appreciate the "spirit" of these regulations, the multiplicity of forms, permits and other red tape is often excessively burdensome to those using these materials in their work.

CONCLUSIONS

The value of frozen tissues, particularly genetic material in the form of gametes and embryos for breeding of vertebrate species, has been reviewed briefly, with special emphasis on current and potential future applications to reptile breeding programs. Realization of the many potential uses for frozen tissues in herpetological studies awaits further research and experimentation.

The division between scientific reality and science fiction is not always clear. Reconstituting formerly extinct life forms is no longer out of the realm of speculation. Rejuvenation of gene pools, increasing the carrying capacity of captive populations, embryo transfers, solving health-related problems, documenting changes within a population over time, matching mating with regard to subspecific status, etc. are all tools currently available and largely accessible (though underutilized) to reptile breeders.

Extinction of wild or captive populations no longer need be FOREVER, if materials are properly collected and preserved NOW. The technical problems must be addressed NOW. If we are to manage captive and wild populations of reptiles and amphibians effectively, we must learn how to take advantage of modern biochemical technology and apply it to herpetology. Over the long term, this knowledge may help solve many problems associated with the preservation and conservation of the earth's herpetofauna.

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