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Zoo Special

Zoos have developed from collections of animals, comparable to stamp collections, in the 18th and 19th century (Artis, the first zoo in the Netherlands, was established in 1838, the Antwerp zoo is the oldest in Belgium founded in 1843, relatively late compared to the Vienna Zoo, the oldest in the world, which opened in 1752) to the institutions they are today. Institutions with a self-declared mission in education, conservation and research.

The role of modern zoos in education is undisputed. As large numbers of people visit zoos (in the Netherlands alone almost 10 million per year), they are in a position to reach a large audience, to which they not only provide entertainment, but also information on natural behaviour of the animals, the importance of protecting biodiversity, threats to the natural habitat etc. A number of species that are nearly extinct in the wild are being kept in zoos, in the hope that the reasons for their dwindling numbers in nature will one day be gone and that with the animals

Zoos offer good opportunities for education, conservation and research of mammals. *Photograph: Jan Vermeer.*

currently kept in captivity, a free-living population can once again be established.

Almost all animals that are being kept in captivity in modern zoos are the result of breeding programmes. The limited numbers of animals necessitate a breeding strategy that is aimed at preventing inbreeding. Animals are exchanged between zoos to keep a viable population, without having to resort to capturing animals in the wild for 'fresh blood'. Keeping zoo animals in shape in their artificial biotope demands a feeding programme that is species-tailored. And apart from all this applied research, animals kept in captivity can even be studied out of curiosity.

Although Lutra is a journal dedicated to mammals that naturally occur in Europe, the role that research on zoo animals plays in conservation and reintroduction programmes merits this special issue. For this issue Frank Princée, (co-)author of several articles, acted as guest-editor, a task he managed to squeeze into his already full schedule for, at first, the Dutch Federation of Zoos and currently as an independent consultant.

Research in zoological gardens

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Abstract: Zoological gardens are important sources of biological knowledge. Zoos played an important role in the development of descriptive biological sciences in the 19th century. To date, zoos apply ethical rules that restrict the use of zoo animals to non-invasive research. Although research in the field of animal behaviour found its way to the zoo, the collaboration between zoos and universities decreased in the second half of the 20th century. Specialisation within biological sciences was one of the causes. Meanwhile, the need for research to solve practical problems in zoo management and conservation increased. The rate of decline in wildlife makes these questions urgent. Fortunately the holistic sciences of conservation biology and zoo biology are now well established and growing. Zoos are actively involved in these fields of research and provide training opportunities as well.

Keywords: conservation, research, training, zoos.

Introduction

Zoological gardens were already historically important sources of biological knowledge. Living examples of numerous species in these institutions were available to 19th century scientists. Zoos therefore played an important role in the development of biological sciences, especially descriptive ones such as anatomy, morphology and taxonomy. The role of zoos in biological sciences has been continued in the 20th century, especially through enabling behavioural studies on non-domestic species (see for example Hediger 1950). The World Zoo Conservation Strategy (WZCS) emphasises the potential source of knowledge that can be gathered in zoos. The availability of this resource to the scientific community lends an intrinsic value to zoo animal collections. Their use is therefore encouraged (IUDZG/CBSG 1993).

Zoos have evolved during the last two centuries from menageries with plain bar cages to nature education centres with naturalistic exhibits and ecodisplays. The overall tendency in modern zoos is to have visitors 'experience' nature. This requires that the behaviour of animals should resemble the species-specific natural behaviour as closely as possible. Consequently, animals need to be housed according to the species social system. In this, the welfare of zoo animals plays an equal role. It is clear that modern zoos offer an even greater potential of knowledge to the scientific community than their 19th century ancestors did.

Early scientists most likely had opportunities to receive biological 'material' from zoos on demand. To date, strict ethical rules apply to research on zoo animals. These rules are not only based on welfare issues but also take into account the fact that many of these animals belong to the category of endangered species. Generally, zoo management would allow only non-invasive research. This means that, for example, blood samples can often only be obtained in cases when an animal is immobilised for veterinary (health) reasons. Behavioural studies that involve manipulation of social structures that potentially can lead to disruptive events will also be subject to ethical rules. Moreover, disruption of social structure can conflict with the educational role of zoos. Research that requires extra logis-

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tics, from extra time of zoo staff (biologists, zoo keepers or veterinarians) to building extra animal holding facilities will also be subject to critical review by zoo management.

It may seem that the various constraints to carrying out research, as described above, are in contradiction to the fact that research is encouraged in the WZCS document (IUDZG/CBSG 1993). The magic word to explain this contradiction is "conservation". The state of nature and its wildlife has dramatically changed during the last century. Various species that are maintained in zoos are endangered or even extinct in the wild. This situation has resulted in a moral obligation of zoos to safeguard these species (animals). Various zoos, for example the members of the Dutch Federation of Zoos, even consider their zoo animal collections as "being given in trust from nature" (Nederlandse Vereniging van Dierentuinen 2000).

Cooperative breeding (or better: population management) programmes for endangered animal species were established by regional zoo associations in the early 1980s (see e.g. Princée 1998). The goal of these programmes is to establish zoo populations that are not dependent on imports from the wild. Furthermore, an equally important goal is to maintain zoo populations as resources for (future) reintroduction (IUCN 1987, IUDZG/CBSG 1993). It is obvious that such programmes do not aim at "simply" keeping animals alive, but require that species are maintained under conditions which "saves" their biological characteristics, from behaviour to genetics.

Information gap

It is estimated that some 6000 species are maintained in zoos world-wide (Flesness et al. 1995). Zoos experience almost daily the enormous gap in biological information on wild populations of these species (see for example Benirschke 1996). Moreover, most information that is available generally refers to the (large) charismatic mammals or non-domestic laboratory species. This enormous gap in biological information not only affects zoos in routine long-term management, but also affects conservation of wild populations equally. Zoos, therefore, tend to facilitate research that is aimed at improving animal management (welfare, husbandry and breeding programmes) and/or conservation of wild populations (Ryder & Feistner 1995, Robinson 1998). It also means that zoos favour applied research rather than fundamental research (Kleiman 1992, Ganslosser et al. 1995).

The historic interest of universities in zoo collections declined in the second half of the 20th century, despite the increased interest from animal psychology and ethology (Hediger 1950). This trend was mainly due to the subdivision of biological science into more specialised topic-oriented research departments. For example, the field of genetics can nowadays be divided over several departments such as Evolution, Molecular genetics and Population genetics. Practical problems in zoos, however, often require an holistic approach. A question like 'Why does this species not reproduce in my zoo?' could require a multi-disciplinary approach which ranges from research on the (sub-)species status and identification, mating behaviour, social structure, nutrition, and health to inbreeding depression. It may be clear that it will be difficult to translate such a question into a research project that fits within the curriculum of one specialised department.

Growth area

Fortunately, new activities regarding research in zoos are increasing, as indicated in contributions to scientific journals such as the International Zoo Yearbook, Zoo Biology and Conservation Biology (Ryder & Feistner 1995). For example, the Dutch Zoo Federation established Stichting Nationaal Onderzoek Dierentuinen (NOD) – or National Foundation for Research in Zoological Gardens – in 1988. An important goal of the NOD is to support the Dutch Zoo Federation zoos in long-term management of animal collections. Research projects that have been carried out by the NOD involved, for example, animal welfare (Griede 1989), juvenile mortality (Debyser 1995), population genetics (Princée 1998) and development of software tools required to register and analyse zoo populations (Princée 1989). Brouwer (1990) and Huizinga (1999) provide an overview of research and student projects in Dutch Zoo Federation zoos.

The active research role of the NOD has changed towards facilitation and co-ordination of projects since the European Association of Zoos and Aquaria has been established. This regionalisation in research is logical as problems that need to be solved in zoo animal management and conservation are not restricted to individual zoos or groups of zoos. However, smaller groups of zoos, such as members of national zoo federations, can often catalyse research.

Resource pool

Zoo animal collections are now, given the conservation context, even more important sources of biological knowledge than they were in the 19th century. Researchers in the fields of animal psychology and behaviour already found their way to the zoo (Hediger 1950). However, research in modern zoos is not limited to these biological sciences. Standardised computerisation of animal records have resulted in a global database system that is maintained at International Species Information System (ISIS) in Apple Valley (MN, USA). This system provides historical data through linking the individual zoo record systems, including parentages, on specimens belonging to some 6000 species which are maintained in zoos world-wide (Flesness et al. 1995). The availability of such data opens an enormous scale of possibilities for research, especially when pedigree data are required.

Basic biological data such as litter size and reproductive life-span can be extracted from this database system. Such data are often not (immediately) available on wild populations, but are required in studies that involve Population and Habitat Viability Assessment (PHVA) of endangered species. Population genetic theory can benefit from the (computerised) population data of zoos. This allows a comparison of results from theoretical models, such as simulation models, with results from empirical data in molecular genetics.

It is clear that killing zoo animals for anatomy, morphology or taxonomy, as happened 150 years ago, is not possible. However, once animals have died (of a natural cause) in a zoo, an enormous source of biological material is potentially available. Any research that requires blood or hair samples of non-domestic animals can obtain such material from zoos. Researchers may have to wait until such samples can be obtained during veterinary control. Therefore, forward planning is important to build a collection of the required samples, before the actual research starts. In this, it must be noted that the Convention on International Trade in Endangered Species (CITES) also applies to parts of (dead) specimens. Therefore, institutions need to have a permit to acquire material from species that are listed under CITES (Nederlandse Vereniging van Dierentuinen 1996).

Zoos provide opportunities to develop and test non-invasive techniques which are applicable to the wild. Increasingly endocrinological and genetic information is being extracted from non-invasively obtained samples of urine and faeces. These techniques can be developed for a wide range of species using zoo populations and can then be applied to wild populations in field studies. Vaccination of wild populations against a specific aggressive viral variant can be required to prevent total extinction of an endangered species. It is important that the effectiveness (protection) of such vaccines are tested before being applied in the wild. Such tests require blood samples that are easier to obtain from zoo animals than from wild specimens (see Schaftenaar, this volume).

Training centre

Researchers who are planning to study animal species in the wild could gain tremendously from

Census techniques for the study of mammals in the wild, such as the identification of individuals on the basis of line pattern in zebra species, can be developed and practised in zoos. *Photograph: Jan Vermeer.*

observing 'their' species in zoos. For example, census techniques as used in assessing size of small populations, may involve identification of individuals on the basis of characteristics such as whisker spots in lions or line pattern in zebra species. Familiarity with the study animals' distinguishing features, postures and behaviour will also help in any habituation process (Williamson & Feistner, in press). It is evident that the more naturalistic the physical and social environment of the captive animal, the better its potential as a model for wild conspecifics. Training in recognising such patterns will be more efficient in a zoo than in the wild. Such capacity development results in more efficient research in terms of time, costs and even quality (better skills of researchers). Given the relatively limited financial resources that are available for conservation such training, like handling laboratory animals, should be included in the curriculum of biological and veterinary sciences.

Conclusion

The zoo world already offers a broad array of research opportunities to scientists who are interested in conservation, but has plenty of potential to offer even more. Since conservation biology is multi-disciplinary, encompassing a blend of basic and applied sciences in biological, sociological and economic subjects, zoos can provide research opportunities to researchers and students in a wide spectrum of disciplines (Primack 1993, Feistner & Price 2000). This volume of Lutra provides some examples of research in Dutch zoos which illustrate the various topics, from genetics to veterinary science, which are part of the wider goal to protect endangered species from extinction.

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Samenvatting

Onderzoek in dierentuinen

Dierentuinen zijn een belangrijke bron voor het verkijgen van biologische kennis. Zij speelden een belangrijke rol in de ontwikkeling van de beschrijvende biologische vakgebieden (zoals anatomie en taxonomie) in de 19e eeuw. Tegenwoordig gelden in dierentuinen ethische regels die alleen non-invasief onderzoek toelaten. Alhoewel ethologisch onderzoek de weg naar de dierentuinen gevonden heeft, nam de samenwerking tussen dierentuinen en universiteiten in de tweede helft van de 20e eeuw af. Eén van de re-

denen hiervoor is specialisatie binnen de biologische wetenschap. In de tussentijd nam de vraag toe naar onderzoek om praktische problemen ten behoeve van collectiebeheer en natuurbehoud op te lossen. De wereldwijde achteruitgang van veel diersoorten maakt deze vragen uiterst urgent. Gelukkig zijn de holistische vakgebieden conservation-biologie en dierentuin-biologie inmiddels gevestigd en groeiend. Dierentuinen zijn actief betrokken bij deze onderzoeksgebieden en bieden tevens mogelijkheden voor training.

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Low frequency calls of captive Asian elephants (*Elephas maximus*)

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Abstract: The aim of this study was to examine the role of low frequency calls in vocal communication of Asian elephants *(Elephas maximus)* in captivity and the possible effects of environmental noise. A classification of two types of low frequency calls was made from 37 hours of audio-recordings of Asian elephants in the Rotterdam Zoo. Behavioural observations suggested that low frequency calls play a role in the social interactions within the family unit. No indications have been found that low frequency calls are used in sexual behaviour, at least not within the context at Rotterdam. The environmental noise in the Rotterdam Zoo is most powerful in the low frequency range (up to 100 Hz). Sources of environmental noise are wind, air-, train- and road-traffic. It is possible, although no direct evidence has been found, that these sounds interfere with vocal communication of elephants.

Keywords: low frequency sound, environmental noise, vocal communication.

Introduction

Although vocalizations and their role in communication have been studied in several groups of mammals, most of this work concerned vocalizations within the range of human hearing. Several mammalian species produce low frequency or infrasonic sounds (i.e. calls below the range of human hearing); for example rhinoceros (Baskin 1992), fin whale (Payne & Webb 1971), and elephant (Payne et al. 1986, Poole et al. 1988). Both Asian *(Elephas maximus)* and African elephants *(Loxodonta africana)* produce low frequency calls, but little is known of the social contexts in which they appear. Poole et al. (1988) made a description of six different types of low frequency calls by wild African elephants and the social contexts in which they appeared. These calls had fundamental frequencies ranging from 14 to 35 Hz. The contexts of these calls suggested that African elephants make use of low frequency calls for the spatial coordination of the group and when searching for mates. Playback experiments supported these observations (Langbauer et al. 1991). It is also known that Asian elephants in captivity produce low frequency calls (Payne et al. 1986). The fundamental frequency of these calls ranges from 14 to 24 Hz, with durations of 10 to 15 seconds. The calls often coincided with the following events: departure and arrival of elephant keepers, daily relocations, and vocal activity of other elephants. Calling elephants were often standing apart from the rest of the group.

An advantage for animals to use calls of low frequency, is that they travel long distances because low frequencies are subject to less environmental attenuation than higher frequencies (Larom et al. 1997). Therefore it has been suggested that elephants use low frequency calls when searching for mates or for maintaining contact between family unit members separated by long distances (Langbauer et al. 1991).

This paper concentrates on low frequency calls of captive Asian elephants and the possible role they play in their communication. In addi-

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Approximately 37 hours of audio recordings were made of Asian elephants by Marcel Muller at the Rotterdam Zoo from February to May 1996. *Photograph: Rob Doolaard.*

tion, attention is paid to possible disturbance of vocal communication due to environmental noise.

Methods

Approximately 37 hours of audio recordings were made of Asian elephants at the Rotterdam Zoo from February to May 1996. Most of the recordings were made between 12 a.m. and 2 p.m., when the elephant keepers were absent (to minimise effects of human presence) and the elephants were in the outdoor enclosure. Some recordings were made in the indoor enclosures between 9 and 10 a.m. Two hours of recordings were made between 6 and 9 p.m. in the indoor enclosures. At the time of observation there were eleven elephants at the zoo, of which six lived together in a group. The group closely resembled a family unit and consisted of two adult females, one of which was the matriarch, three young females (ten, six and one years old) and one young male (seven years old). The other elephants (one adult male and four adult females) were housed in enclosures separated from the group in changing combinations of individuals. The adult male was in musth during part of the observations. Some of the females were in oestrus during several days of our observations. During this period several copulations took place.

The recordings were made on a Nagra IV SJ recorder with a Bruel & Kjaer 4155 microphone, mounted on a Nagra QJPA microphone preamplifier. Recordings were made at 3.81 cm/s recording speed and were analysed and sonographed at 19.05 cm/s, with the sound analysis programme Signal 2.23 (Engineering Design, Belmont, USA). Behaviour, audible sounds and movements in the enclosures were also noted during sound recording sessions.

Recordings were made at variable distances of at least five metres from the elephants. The following sound parameters were measured from the sonograms: 1. *Dominant frequency*: The frequency of highest intensity as manifested by the darkest part of the sonogram. The measurement was taken at the midpoint. 2. *Fundamental frequency*: The lowest frequency of a sound. This frequency was determined by measuring the difference between the harmonics as displayed on the sonogram. 3. *Peak frequency*: The highest frequency of a sound as displayed on the sonogram. 4. *Duration*: The time, in seconds, from the onset to the end of the sound as displayed on the sonogram (Berg 1983).

Results

A total of over 100 different vocalizations were recorded. Amongst other vocalizations (for example trumpets) 17 calls containing components of low frequency were recorded. These could be classified into two types: 1. *Low frequency calls* (figure 1). Defined as long, sometimes modulated, rumbling calls (up to 22.5 s) with low fundamental frequencies. The mean fundamental frequency found for eleven recordings of these calls was 39.5 ± 23.7 Hz, ranging from 17.6 to 88.0 Hz. The mean peak frequency was 161.9 ± 63.3 Hz (ranging from 88.0 to 297.7 Hz). The mean dominant frequency ranged from 25.8 to 195.0 Hz with a mean of 106.9 ± 71.6 Hz. The values of different individuals showed overlap. All of the eleven calls were found in the context described below. Some of the calls were of very low intensity. 2. *Roar* (figure 2). This call begins as a high intensity scream (a), comparable to a trumpet, with a mean fundamental frequency of 799 ± 140 Hz, then progresses into a low frequency call (b), with a mean fundamental frequency of 59.8 ± 59.2 Hz, lasting up to 5 seconds. The roar was recorded six times in one individual (one of the young females) only.

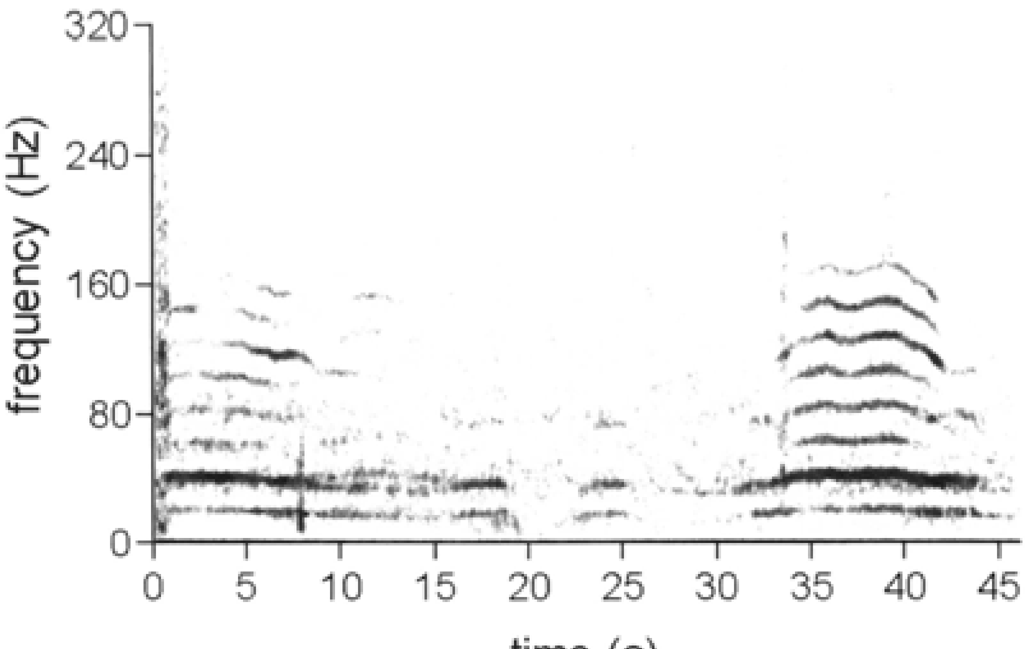


Figure 1. Sonogram of two low frequency calls produced by one of the adult females of the group with fundamental frequencies of 17.6 and 20.4 Hz.

Low frequency calls by Asian elephants play a role in the spatial coordination of the group.
Photograph: Jan Vermeer.

A sequence of different vocalization types was observed five times. Each sequence started with a trumpet or a roar and was followed by a combination of trumpets, roars and low frequency calls (figure 3). These sequences, emitted by a group member standing several metres apart from the rest of the group, lasted up to two minutes and always contained low frequency components. The rest of the group made trumpets and low frequency calls in response, while moving in the direction of the calling individual to stand close to each other, with the one year old female in the centre of the group. The group continued vocalizing for 3 to 4 minutes after gathering together.

Although the male was in musth and some of the females were in oestrus during part of the study, no indications were found that low frequency calls play a role in reproductive social interactions.

The Rotterdam Zoo is situated near the centre of Rotterdam, close to a railway station and an airport, both sources of noise that could possibly disturb vocal communication between the elephants. Wind is another source of noise that obscured infrasound recordings made outside. Less than 50% of the calls that were recorded, could be used to describe the different types of vocalizations. The other calls were difficult to analyse because of obfuscating noise. The noise found on our recordings was loudest in the lowest frequencies (up to 100 Hz). A reaction to external noise was observed on one occasion. One of the group-females vocalized and pointed with her trunk in the direction of a passing truck. Analysis of the recordings of the sound produced by the truck showed a more or less harmonic structure with a fundamental frequency of 50 Hz and a peak at about 100 Hz. This is similar to the structure of low frequency calls produced by the elephants.

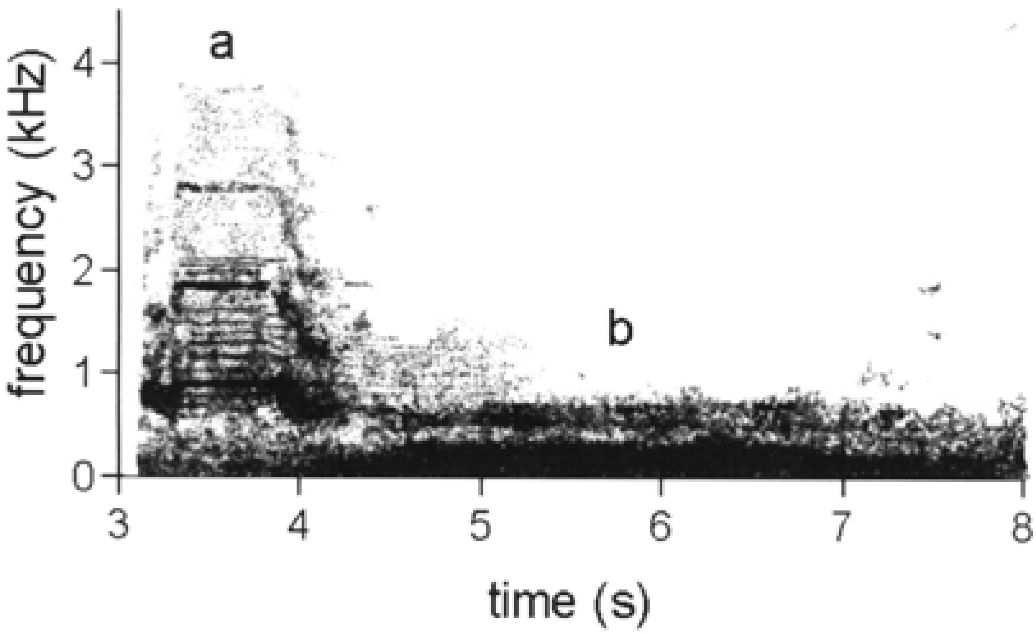


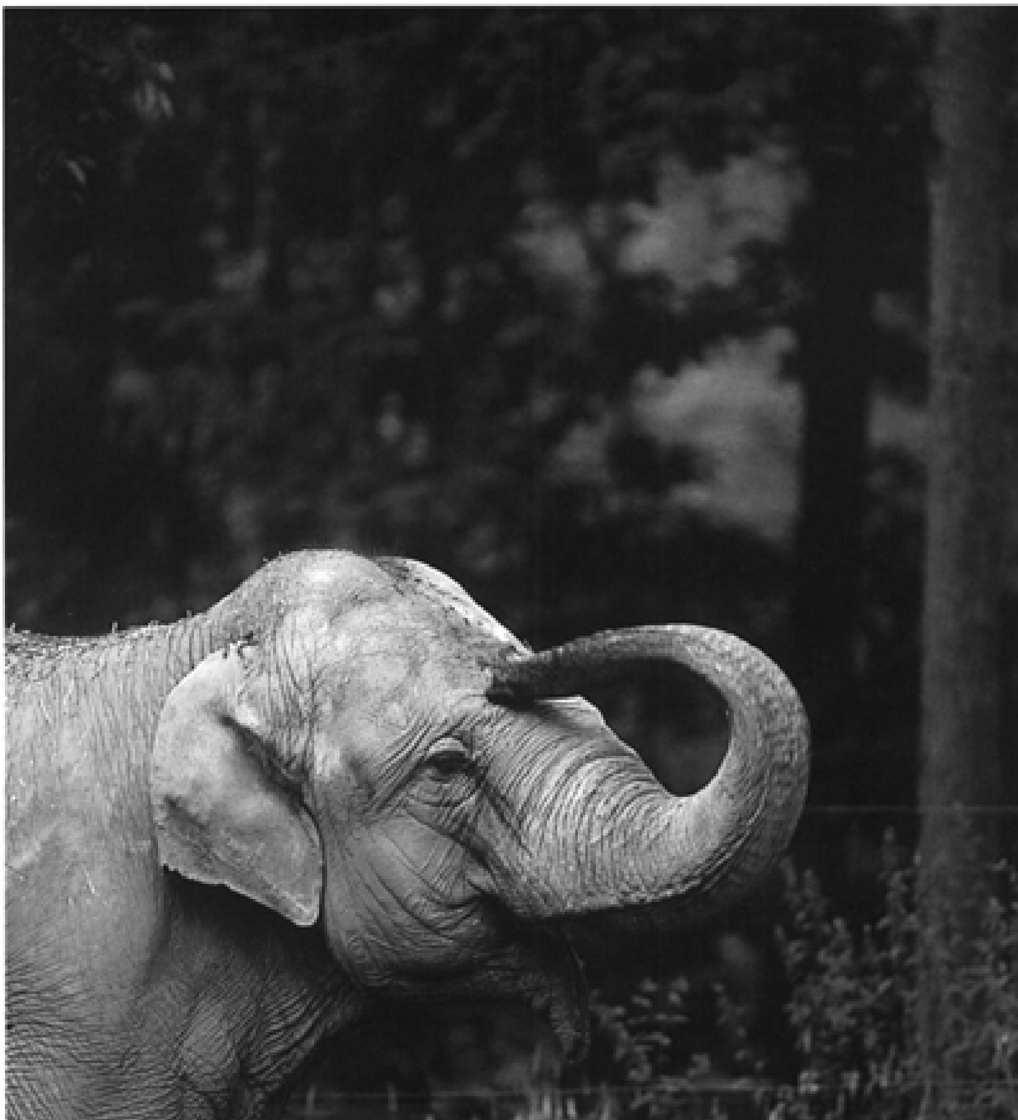
Figure 2. Sonogram of a juvenile female roar with fundamental frequencies of 937.0 and 70.2 Hz. The sonogram displays a high intensity scream between 3.2 and 4.3 seconds, and a low frequency call between 4.3 and 6.8 seconds.

Discussion

The two different types of low frequency calls identified from our recordings were comparable in structure, duration and (fundamental) frequencies to some of the low frequency calls recorded by Payne et al. (1986). Like Payne et al. (1986), we found that low frequency sounds were mostly made by elephants standing at some distance from other individuals. The responses to the calls suggest that they play a role in the spatial coordination of the group, similar to what has been found in African elephants (Poole et al. 1988).

In contrast to Poole et al. (1988) we found no indication of exceptional calling activity relating to musth, although the male in the group was in musth for some days during observations. In general the call rate in our group was much lower than reported by Payne et al. (1986), suggesting that housing conditions might affect the call rate, e.g. elephants in the Rotterdam Zoo stand close together, so infrasounds are not necessary for communication. However, our recordings were mostly made around midday, whereas most calling might occur later during the day or at night.

Studies on bird vocalizations have suggested that vocal communication might be disturbed by environmental noise, as changes in sound environment have been coupled to changes in song repertoire (Lehtonen 1983). Birds and presumably mammals as well, have various ways of detecting signals in a noisy environment (Klump 1996). Nevertheless, environmental noise may possibly interfere with elephant communication because of the low intensity of the low frequency calls. Environmental noise in the Rotterdam Zoo is highest in the lowest frequencies, which may be unlike environmental noise in the elephants' natural habitat. The reaction of one female to a localized noise source (a passing truck) indicates a potential effect of such noise. Therefore the influence of environmental noise on vocal communication in elephants and other zoo animals requires further study.



Observations suggest that environmental noises might interfere with low frequency vocal communications of elephants. *Photograph: Jan Vermeer.*

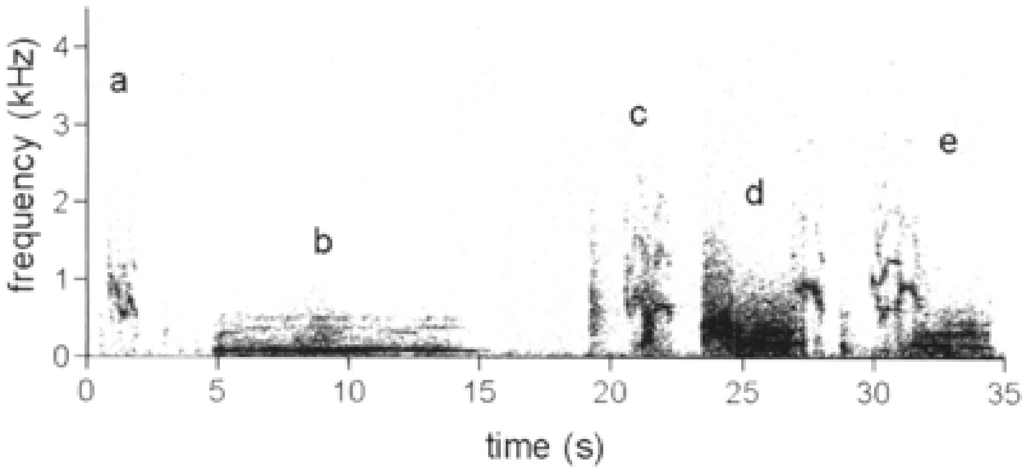


Figure 3. Sonogram of a sequence of different vocalization types recorded in the evening in the indoor enclosure. It was preceded by the one year old female of the group screaming (a = trumpet; b = low frequency call; c = trumpet; d = roar; e = roar).

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Samenvatting

Infrasone communicatie bij de Aziatische olifant (*Elephas maximus*) in gevangenschap

Het doel van dit onderzoek was, meer te weten te komen over infrasoon geluid in de vocale communicatie van de Aziatische olifant (*Elephas maximus*) in gevangenschap en de mogelijke gevolgen van omgevingsgeluid. Een classificatie van twee soorten infrasoon geluid werd gemaakt uit 37 uur geluidsopnamen van de Aziatische olifanten in diergaarde Blijdorp in Rotterdam.

Gedragswaarnemingen suggereren dat infrasoon geluid een rol speelt in de sociale interactie binnen de familiegroep. Er zijn geen aanwijzingen gevonden dat infrasoon geluid gebruikt wordt in sexueel gedrag, tenminste niet binnen de context van diergaarde Blijdorp in Rotterdam. Het omgevingsgeluid, zoals veroorzaakt door trein-, auto-, en vliegverkeer is het sterkst in de lage frequenties (tot 100 Hz). Het is mogelijk dat deze geluiden de vocale communicatie bij olifanten verstoren, hoewel hier geen duidelijk bewijs voor gevonden is.

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Vaccination of non-domestic carnivores: a problematic obligation

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Abstract: Infectious diseases pose a risk to non-domestic captive carnivores. Since commercially available vaccines are not always safe for non-domestic species, they cannot be used without testing them for safety and efficacy. A short list of diseases for which vaccination is recommended is provided. Reintroduction programs should include studies of the need to protect the animals, prior to their release. Recently, protocols for the immunisation of carnivores in reintroduction programs have been established.

Keywords: vaccination, infection, carnivores, reintroduction.

Introduction

In the last decade, the number of safe vaccines available for domestic carnivore species has decreased. Modified live vaccines that are not safe for non-domestic species have replaced the killed vaccines.

Few people in the western world remember the symptoms of canine distemper and rabies in the domestic dog or foot-and-mouth disease in cattle, while these infections are still regular visitors of African and Asian countries. Since most of the domestic pet animals in the western world are well protected against the major viral infections, animal keepers may get the wrong safety feeling that their animals do not run any risk of attracting these often fatal infections. Captive animals in zoological collections are just as susceptible to infectious diseases as are their free ranging relatives. However, the infection pressure in captive situations is generally lower, resulting in a lower incidence of outbreaks of infectious diseases. Feline panleukopenia used to be a common infection in captive felines (Appel 1987). Parvovirus infections have been diagnosed in captive maned wolves (*Chrysocyon brachyuris*, Fletcher 1979). Several big cats in North American zoos have died from canine distemper (Appel et al. 1994). In the past decade, the Rotterdam Zoo lost a European otter (*Lutra lutra*) that had died of a parvo-infection and at least one Oriental small-clawed otter (*Aonyx cinerea*) due to canine distemper (W. Schaftenaar, unpublished data).

The relatively low infection pressure in zoos is the result of several factors: 1. Vaccination strategies for domestic animals lower the incidence of diseases in that area. 2. Proper fencing of zoos reduces the risk of intrusion by non-vaccinated stray animals. 3. Many zoos have adopted quarantine protocols for incoming animals. These measures may reduce the import of infectious agents, but do not provide a watertight protection. Stray cats and dogs may still enter the zoo through weak points in the fences; small free-ranging carnivores like raccoons, viverrids or mustelids can not be kept out of the zoo. Waterfowl and other birds have free access to almost every location. In many zoos mice represent the biggest part of the mammal collection.

Commercially available vaccines

The major part of the commercially available vaccines consists of vaccines against virus infections developed for domestic animals. Canine distemper virus (CDV), rabies and feline panleukopenia or parvovirus (FPV) are well-known examples.

Vaccines can be divided into live and inactivated or killed vaccines. With the aid of modern technologies live vaccines can be attenuated in a way that makes the vaccine-virus harmless to the host animal, but still potent enough to reproduce in this animal. The advantage of live vaccines is the formation of large amounts of virus antigen as a result of the virus replication. In general this will induce a better immune response from the host, which lasts longer than when inactivated vaccines are used. Live vaccines are specifically developed for single species, while inactivated vaccines generally speaking can be used for multiple species. In the past, the use of live, attenuated vaccines against canine distemper, developed for domestic dogs has resulted in death in a number of species, like the red panda (*Ailurus fulgens*, Bush et al. 1976), black-footed ferret (*Mustela nigripes*, Carpenter et al. 1976), gray fox (*Urocyon cinereargenteus*, Halbrooks et al. 1981), kinkajou (*Potos flavus*, Kazacos et al. 1981), South American bush dog (*Speothos venaticus*, McInnes et al. 1992), European mink (*Mustela lutreola*, Sutherland-Smith et al. 1997), maned wolf (Thomas-Baker 1985) and Fennec foxes (*Fennecus zerda*, T. Martens, unpublished data). Apparently, in these species the attenuated vaccine virus can escape the defence mechanism of the host and regain its virulence. Therefore, live virus vaccines should not be used in species for which they were not developed, unless vaccination trials have proven its safety for that particular species.

Panthers are susceptible to several viruses that can also affect domestic cats. Besides, canine distemper has been found to be a pathogen to several big cat species. *Photograph: Jan Vermeer.*

New vaccine generations

In recent years, new generations of vaccines have been developed. The subunit vaccine with CDV Immuno-Stimulating-Complexes (ISCOM) is an experimental, inactivated vaccine based on elements of the mantle of the virus. This type of vaccine is harmless for the host, since virus replication cannot take place. It was developed for harbor seals (*Phoca vitulina*) in the North Sea to protect stranded animals against phocine distemper (Visser et al. 1992). Since 1993 the vaccine has been used experimentally in red pandas in several European zoos. However, the immune response is not very promising (W. Schaftenaar, unpublished data).

Another recently developed type of vaccine is a recombinant canary pox-vectored CDV-vaccine. In this vaccine, part of the genetic material of the virus is incorporated in a canary poxvirus. Mammals are dead-end hosts for this virus. When the virus has entered the host cell, its DNA that also contains information for the production of CDV-antigens gets the opportunity to produce

Small-clawed otter, a species that is susceptible for canine distemper. Rotterdam zoo lost a specimen to this virus. *Photograph: Edgar van der Grift.*

antigen material of the distemper virus, which induces immunity against CDV. Recently, such a canary pox-vectored CDV vaccine was released on the American market (Merial Ltd, Athens, Georgia 30601, USA) for use in ferrets. Unpublished data from Montali demonstrated its potential value for use in red pandas. In black-footed ferrets x Siberian polecat (*Mustela eversmanni*) hybrids it produced a good immune response (Williams & Montali 1998).

Neither of these new types of vaccines has been widely tested for use in different species. Harbor seals and ferrets challenged with CDV after vaccination with the CDV-ISCOM-vaccine could be sufficiently protected by this vaccine. However, 47 out of 50 African wild dogs (*Lycaon pictus*) died of a natural canine distemper infection despite the repeated administration of the same vaccine over several years (A.M. Visee, unpublished data).

Recommendations for non-domestic species

A list of diseases for which vaccination is recommended is shown below (Fowler 1986): 1. *Canine distemper virus* – Most canidae, hyaenidae, some large cat species, mustelidae, viverridae and procyonidae are susceptible. The commercially available vaccines are modified live vaccines and not suitable for non-domestic species. The recently developed canary pox-vectored vaccine for ferrets seems to be promising. Zoos have to work together with research institutes to develop a safe vaccine for a wide range of species. To reduce the risk of cross-infection, domestic dogs that might come into contact with non-domestic species should be vaccinated against CDV. 2. *Parvovirus* - Canine parvovirus (CPV) and feline parvovirus (FPV) are important pathogens for non-domestic carnivores (ca-

Fennec fox, a species that cannot be vaccinated with a (modified) life distemper vaccine. *Photograph: Jan Vermeer.*

nidae, hyaenidae, felidae, procyonidae, mustelidae and viverridae). Unfortunately the killed CPV-vaccine has been withdrawn from the market in several countries. The efficacy of a killed FPV-vaccine for canidae has not yet been studied. 3. *Rabies virus* - In countries where rabies is indigenous, contact between wild animals and non-domestic zoo species should be avoided. If this cannot be achieved, vaccination of these animals should be practised. 4. *Infectious rhinotracheitis virus* - Only felidae are susceptible to this virus. Non-domestic members of this family should be regularly vaccinated with a killed vaccine. 5. *Leptospirosis* - Few data are available about this bacterial disease in non-domestic species. Vaccination with a killed vaccine has been recommended in canidae, hyaenidae, procyonidae, mustelidae and viverridae (Fowler 1986). 6. *Feline Leukemia virus* - Although there is evidence that many non-domestic feline species are susceptible to one of the numerous strains detected, vaccination is not recommended for two reasons: serological screening will be biased by vaccination and efficacy has not been proven (Kennedy-Stoskopf 1999).

Vaccination of free ranging carnivores

Vaccination of free ranging animals has long been under discussion. In general, vaccination of free ranging animals is not justified, since natural selection is an important mechanism to maintain healthy populations. However, when captive or rehabilitated animals are released to the wild, they may miss the acquired immunity of their free ranging conspecifics. For several years stranded harbor seals in The Netherlands have been vaccinated with a CDV-ISCOM-vaccine prior to release to the wild in order to protect the released animals against phocid distemper that was endemic in the North Sea.

Human presence may pose a serious threat to free ranging populations. The decrease of biotopes where these populations can live undisturbed, increases the contact between wild and domestic animals. Viral infections in domestic species have become a serious problem for free ranging animals. CDV has played an important role in the extinction of the black-footed ferret in the USA. In the conservation programme for this species in the USA, much attention is paid to the development of a safe CDV-vaccine for this species (Williams & Thorne 1999). Failure of any reintroduction programme of ferrets is guaranteed if the animals involved are not protected against CDV. In most parts of Africa the African wild dog population is threatened by decline of their natural habitat and infectious diseases like rabies and CDV. Antibodies against CDV have been demonstrated in free ranging animals (Alexander et al. 1993). A vaccination programme for rehabilitation of this species should include rabies and CDV.

IUCN-guidelines for health screening protocols prior to release of captive animals into the wild have been published recently (Woodroft 2002). The lack of reliable vaccines makes this a difficult process and forces the institutions involved in these programmes to co-operate in the development of safe vaccines (Montali et al. 1998).

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Samenvatting

Het vaccineren van niet-gedomesticeerde carnivoren: een verplichting met problemen

Infectieziekten vormen een risico voor niet-gedomesticeerde wilde carnivoren. Aangezien commerciële vaccins niet altijd veilig zijn voor niet-gedomesticeerde diersoorten, kunnen deze niet zonder voorafgaand onderzoek naar de veiligheid en werkzaamheid worden gebruikt. Een korte lijst van ziektes waartegen gevaccineerd zou moeten worden, wordt gepresenteerd. Recentelijk is door de IUCN een protocol gepubliceerd dat richtlijnen geeft voor immunisatie van carnivoren die in het kader van een herintroductieprogramma weer zullen worden losgelaten.

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Forwards in zoo nutrition

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Abstract: In the 1980s, zoo animal nutritionist became an acknowledged profession. Since then, an increasing number of animal nutritionists were employed by zoos all over the world. Essential for a zoo animal is the establishment of a nutrition programme. The Zoo Animal Matrix developed by the Brookfield Zoo, gives an overview of factors influencing zoo animal nutrition. The development of a good zoo animal diet supported by nutritional research is not an easy task. It should be achieved by a combination of practical and scientific input. Nowadays, many zoos still feed their animals according to the so-called Cafeteria-Style Feeding, which means that it is doubtful whether the animals get a nutritional balanced diet. The current need to improve zoo nutrition is illustrated by the fact that target projects of zoos (European Endangered species Programmes or EEPs) still do not include adequate nutrition sections in their husbandry guidelines. Since a few years, specialized groups like the Nutrition Advisory Group (NAG) and the European Zoo Nutrition Research Group (EZNRG) focus on improving zoo animal nutrition. Their targets and priorities will be a stimulus to further professionalise zoo animal nutrition.

Keywords: nutrition, research, NAG, EZNRG, EEP, Cafeteria-Style Feeding, zoo matrix.

Historical perspective

In the nineteenth century the importance of major food constituents such as protein, fat, carbohydrate, fibre and mineral nutrition was recognized. In the twentieth century the importance of vitamins, fatty acids, amino acids and several trace elements were discovered. However, many interactions between these components still have to be established. The first records concerning zoo diet information were published by the Calcutta zoo by Sanyal (1892). In 1888 Bland Sutton published information about severe metabolic bone diseases in zoo species (Dierenfeld 1997). During the 1920s and 1930s Dr. Ellen Corsen-White studied primate diets in the Philadelphia zoo. She introduced the recipe known as "Zoo Cake" which was designed with commercially available products (Crissey 2001a). In 1937 Dr. Herbert Ratcliffe started to improve diets of zoo animals in Philadelphia Zoological Garden. Rapid scientific nutritional advances in livestock, fur-, pet-, and laboratory animal feed industry helped Ratcliffe to develop diets such as mixed meat rations and a composite herbivore ration. In the 1960s Wackernagel developed herbivore pellets for grazing and browsing species, omnivore cakes and pellets, supplemented meat mixtures for carnivores, soft-bill bird feeds, pelleted poultry rations for ducks, geese, cranes and ostriches and colour feeding rations for flamingos. The nutrient compositions developed by Wackernagel were primarily based on the nutrition knowledge from animal agriculture at that time (Dierenfeld 1997).

The 1966 and 1976 editions of the International Zoo Yearbook focused on zoo animal nutrition. Zoo veterinary books also paid attention to diets for zoo animals (Dierenfeld 1997). From 1970 until 1988 the Institute of Zoology of the Chicago Zoological Society performed nutritional studies. Scientists from this society, interested in nutrition, developed the Nutrition Society (Crissey 2001a).

Although several zoo staff members, for example of Dvur Kralove Zoo, Jersey Zoo and Rotterdam Zoo, were in contact with institutions that aimed to improve diets; it was not until the mid-seventies that professionally trained nutritionists were hired. Dr. Duane Ullrey, professor emeritus at Michigan State University, is considered the modern-day mentor of zoo nutrition and nutritionists in North America. In Europe the first zoo nutritionists were employed in the 1980s. In 1994 the American Association of Zoos and Aquariums recognized the value of scientific advice on nutrition. During that time the Nutrition Advisory Group (NAG) was founded. It took until 2001 before the European Association of Zoos and Aquaria (EAZA) recognized the European Zoo Nutrition Research Group (EZN-RG) as a specialist group in zoo animal nutrition in Europe.

Nutrition in zoos and its implications

Building a nutrition programme in zoos

Marquès (1999) argues that, before starting to work on nutrition in zoos, a plan for the foundation and structure of the nutrition service should be defined. Nutritional problems are sometimes not detected in time which means that the advantages of a nutritionist may not been recognized immediately. When building a nutrition service, the advantages from both the perspective of the animals and from the institutions should be presented in order to gain financial and moral support. According to Marquès (1999) it is furthermore important as a nutritionist to become familiar with the goals of the organization and all aspects associated with diet preparation and distribution. If these aspects are missing, a nutritionist will have difficulties in developing a good nutrition service.

Zoo animal nutrition matrix

An appropriate diet for captive exotic animals is affected by many factors. This is illustrated by the 'zoo animal nutrition matrix', which was recently developed by Crissey (2001b; figure 1). It is divided into four sections: 1. *Consumption* of a diet, which is affected primarily by the client's wishes and animal preferences. 2. Nutritional *requirement*, which should be considered as species specific. 3. The *health* factor, which is primarily affected by infectious as well as non-infectious agents, preventive health measures and by either general ideas on nutrition or trial and error experiences of veterinarians. 4. The *management* factor. Important parts of management are food storage capacity, environmental factors, the enclosure, and the feeding regime.

Research projects

Because zoos can rarely offer diets which exactly match their animals' natural diet, substitutes have to be developed. This can be done by trial and error studies or by scientific studies on the digestive characteristics and physiology of an animal.

Many diets in zoos are based on tradition and developed by trial and error. Animals may seem to do well and even reproduce on such diets. However, diet-related diseases still frequently occur in zoo animals and almost no information is available on the effects of balanced diets on health and longevity (Hatt 1999).

Nutritional study projects are often based on a vaguely defined question. For instance, when post-mortem studies do not reveal a clear cause of death, the involvement of a nutrition related problem is often assumed. However, in most cases the suspected nutritional problem is not clearly described. In order to do nutrition research it is vital that the nutritional problem is exactly described.

In contrast with nutritional experiments in a laboratory setting, the conditions for zoo animals used for research are not standardized: mostly only a few animals can be used, the animals differ in age and the husbandry is not controlled. No reliable statistical analysis can be done on the results. Weather conditions, animals' health and seasonal changes may vary and influence the results. Behavioural and biological characteristics may also nullify the results of many research

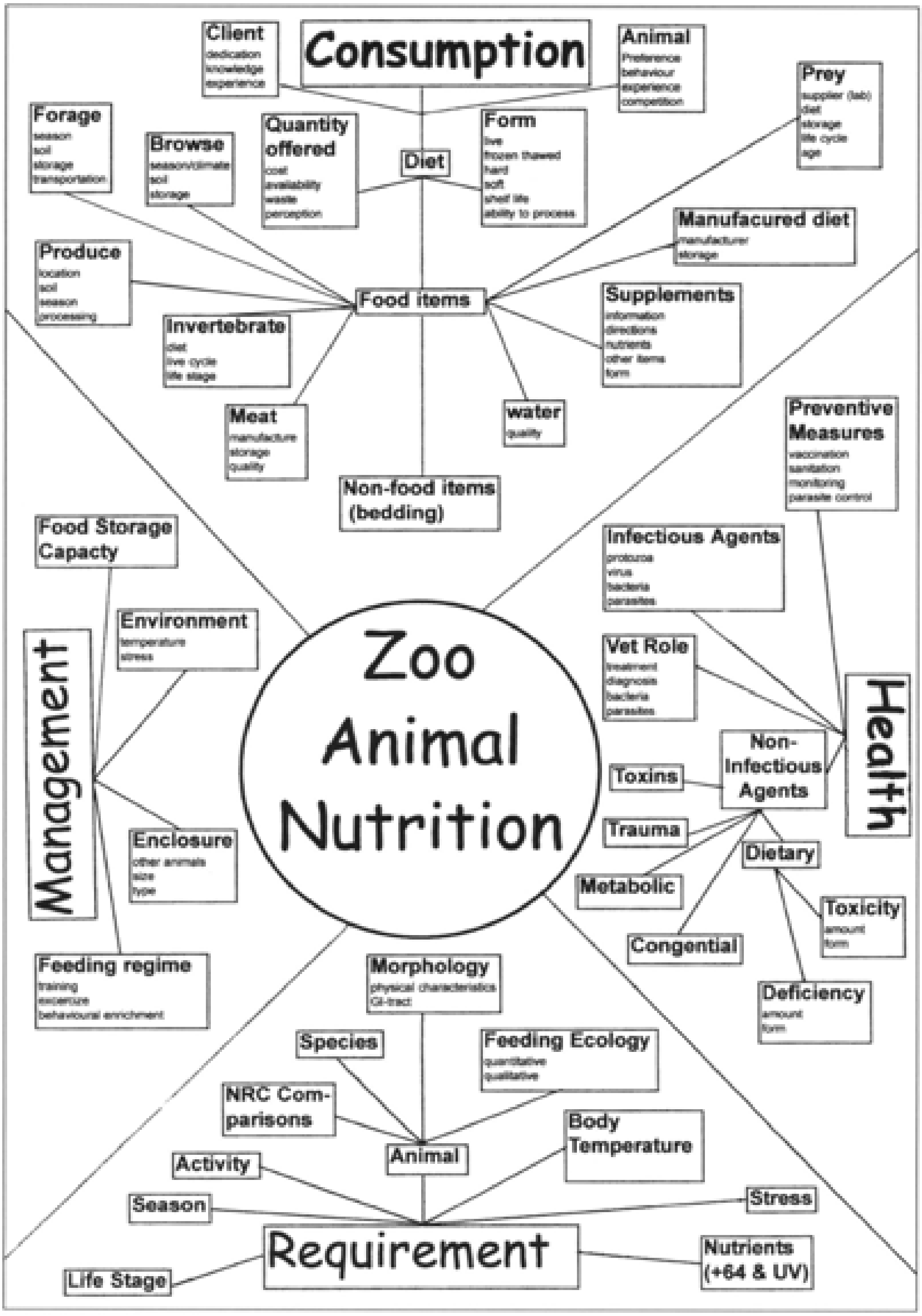

Figure 1. Zoo Animal Nutrition Matrix developed by Crissey (2001b).

Since the 1980s zoo animal nutrition is an important issue in zoo management. *Photograph: Jan Vermeer.*

projects. Researchers should therefore be prepared for several modifications in the experimental design (Hatt 1999).

Furthermore, invasive procedures are usually not possible. Most nutritional information has to be gathered from easily accessible samples like faeces, urine and food, although even collecting a simple food sample is not always easy. Food items on diet sheets are mostly not quantified and can be arbitrary. Every animal keeper has their own interpretation of a diet sheet. Food used for enrichment is often not included in the diet sheet. There may also be a considerable degree of seasonal variation in the diet offered and consumed (Bond 2001). Especially when more individuals of the same species are housed together, it is nearly impossible to figure out how much food is eaten by each animal. And in the situation where more species are housed together, calculations of food amounts eaten are even less reliable.

Collaboration of zoos and institutes like universities is needed to improve zoo animal nutrition. Nutritional studies should be a combination of the practical input from zoo staff and the scientific background of the skilled staff of the institute. Data from research projects should be published even if the results have no statistical significance, as long as the project is important (Hatt 2000). In Rotterdam Zoo for example, digestion tests showed that colobines can digest fibre. By increasing the amount of fibre the faeces' consistency improved as well. Besides these tests an extensive literature survey was made. The survey showed that browse consumed by colobines in their natural habitat has a much higher amount of fibre than the browse particles commonly offered in West European zoos. Therefore a special pellet was developed with a very high amount of fibre in order to compensate for the lack of fibres in the diet compared to their natural diet (Nijboer 1996, Nijboer 2001).

Cafeteria-Style Feeding

In many cases the diets of zoo animals are offered according to a method called "Cafeteria-Style Feeding" (CSF). The principle is to offer a variety of food of which the animals can make their own choice what to eat. For wild animals it is shown that there may be a certain degree of 'nutritional wisdom' for a few nutrients. If animals in captivity would have the capability to eat a balanced diet out of a CSF offered diet, nutritional problems would not be expected. However, several studies show that if a CSF diet is fed many times, it may result in dehydration or obesity of the animal (Marquès 2001).

Animals in captivity cannot consume the complex and seasonal diet which they get in the wild. CSF does not solve this problem, since the animals cannot make the right choices. Other factors which influence the food selection are physiology and morphology of the intestinal tract, taste, texture and size, and colour of ingredients. Because animals are not living in their natural habitat, changes in their behaviour may occur, resulting in an unnatural feeding behaviour and wrong food choices when offered a CSF diet. Psittacines for example have individual preferences for foods based on previous experience, food placement, particle size, fat content, texture, shape, colour, and taste. These preferences can be very strong in these birds and may lead (if not discouraged by the owner) to a feeding behaviour that is most readily described as an "addiction" to certain food items, like sunflower seeds or peanuts. This type of CSF feeding can result in severe nutrient deficiencies if the selected food is not nutritional-complete and balanced (Brue 1994).

A CSF diet is often used in zoos because it is supposed to stimulate the natural feeding behaviour of animals. This assumption should be considered with care: in the Rotterdam Zoo tests have shown that parrots only feed 20 minutes more a day on a CSF diet, than on a pellet based diet. It is suggested that a balanced diet should be preferred, where the animals can make their choice to a limited degree (Brue 1994).

The status of nutrition sections in EEP husbandry guidelines

In most modern zoos breeding programmes have been developed for endangered species. Developing breeding programmes is considered by many zoos as one of their key targets. In Europe these programmes are called European Endangered species Programmes (EEP) and are co-ordinated by an EEP co-ordinator. One of the tasks of an EEP co-ordinator is to develop husbandry guidelines in order to achieve optimal conditions for well-being and reproduction.

These guidelines should contain a section on feeding which summarises the consumed diet *in*

Based on research of their natural diet, pellets were developed with high amounts of fibres for Southeast Asian colobines in Rotterdam Zoo. *Photograph: Jan Vermeer.*

Cafetaria-Style Feeding is often used in zoos because it is supposed to stimulate the natural feeding behaviour of animals. However, studies show that not all animals in captivity have the capability to select a balanced diet. *Photograph: Jan Vermeer.*

situ and *ex situ* in terms of nutritional composition. The nutrition section should also contain information on special dietary requirements, hand rearing protocols, nutrition-related health problems, and a bibliography. A questionnaire was sent out by Nijboer in 2000 to all EEP co-ordinators in order to find out how many nutrition sections within the EEP guidelines were published. 66% of the questionnaires were returned. 48 of the EEPs contained published guidelines and 31 of the guidelines contained a nutrition section (table 1; Fidgett et al. 2001). Although zoos currently focus much of their research, conservation and husbandry efforts on target species, still many husbandry guidelines have to be published and still many more contain an inadequate nutrition section. Since the nutritional information on target species in zoos is insufficient, it is likely that our knowledge of the remaining species is even worse.

The way forward

The Nutrition Advisory Group (NAG) of the American Zoo and Aquarium Association (AZA) was established in 1994. Its primary goals are to identify nutritional and dietary problems in zoos and to facilitate solutions to these problems. Bi-annual nutrition conferences are organised to discuss the progress of nutrition research. Fact sheets containing updated information on specific zoo nutrition items are regularly published. Advice is given by the members of the NAG to the North American Species Survival Plan (SSP) and Taxon Advisory Groups (TAG), considering nutrition related problems and to set up nutritional guidelines within the husbandry guidelines (Crissey 1999).

Furthermore, the Comparative Nutrition Society (CNS) was established in 1994. During bi-annual meetings their research projects are dis-

Table 1. The structure of the nutrition section within EEP husbandry guidelines (Fidgett et al. 2001).

Subject	Number
Guidelines published (% of all programmes)	48 (38%)
Guidelines with nutrition sections	31
Nutrition section presents:	
Feeding schedule	24
Nutrient content of diet	19
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cussed. More than the NAG, this group focuses on fundamental nutrition research, related to exotic animals.

In 1999 the First European Zoo Animal Nutrition Conference was held in the Rotterdam Zoo. The success of that conference was a stimulus to organize a second conference to be held in Marwell Zoo in 2001. Unfortunately due to the fear of foot & mouth disease the conference was cancelled. The next official meeting on European zoo nutrition was in September 2001 where the Research Committee of the EAZA supported the funding of the European Zoo Nutrition Research Group (EZNRG) and the funding of the European Zoo Nutrition Centre (EZNC). The EAZA Research Committee recommended the computer programme *Zootrition*™, developed by the Wildlife Conservation Society in New York, as the standard software for nutrition programmes in European zoos.

The priorities of the EZNRG are: 1. Establishing a bibliographic database of nutritional references relevant to European TAGs which will be published on the internet. 2. Develop a zoo nutrition education package suitable for all zoo staff, from animal keeper level upwards, covering elementary aspects of nutrition, diet collection and promoting the use of *Zootrition*™ software. 3. Contact TAGs for husbandry guidelines, offer to review their nutrition section and make suggestions on how they can standardize the best diets over a period of time. 4. Find external funding and sponsorship for the EZNC. The EZNC started in January 2002 and will help the EZNRG to explore the priorities. It is expected that both EZNRG and EZNC will be a stimulus in the further development of zoo animal nutrition in Europe.

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Samenvatting

Vooruitgang in de voeding van dierentuindieren

Pas sinds de jaren tachtig en negentig hebben dierentuinen meer medewerkers aangetrokken om zich full-time met voeding bezig te houden. Om een adequaat voedingsprogramma te ontwikkelen moet aan vele factoren worden gedacht. De "zoo matrix" geeft aan met welke factoren. Het is bijna onmogelijk om verantwoord wetenschappelijk voedingsonderzoek uit te voeren in dierentuinen. Vaak moeten concessies worden gedaan aan de randvoorwaarden voor het onderzoek. In het algemeen kan gesteld worden dat er nog weinig uniforme voedingsrichtlijnen zijn opgesteld voor dierentuindieren. Organisaties zoals de Nutrition Advisory Group (NAG) en de European Zoo Nutrition Research Group (EZNRG) zetten zich in om dat te verbeteren.

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Genetic management of small animal populations

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Abstract: European zoos, associated in the European Association of Zoos and Aquaria (EAZA), established co-operative breeding programmes for zoo populations in 1985. European Endangered species Programmes (EEPs) and European Studbooks (ESBs) for some 250 species have been established as at March 2002. The goals of these programmes are to maintain viable zoo populations without (or with limited) import from the wild. These populations can serve, whenever feasible, as sources for reintroduction of endangered species. Retention of genetic variation in populations of endangered species is a prerequisite for their future survival. This means that conservation efforts should also include genetic management of zoo populations, especially when reintroduction is part of a conservation programme. Therefore, maintenance of sufficient levels of genetic variation and minimising inbreeding are important objectives of zoo breeding programmes. Population biologists who work for zoological gardens have developed various strategies, models and computer tools to assist zoo biologists in the co-operative management of zoo populations.

Keywords: population management, breeding programmes, genetic variation, studbooks.

Introduction

Zoological gardens started breeding programmes for the European bison or wisent (*Bison bonasus*) and Przewalski's horses (*Equus przewalskii*) at the beginning of the 19th century. These propagation programmes resulted in reintroduction of the European bison in Bialowieza Primeval Forest as early as 1929 (Pucek 1991); and reintroduction of the Przewalski's horse in Mongolia in 1992 (van Dierendonck & Wallis de Vries 1996).

Management strategies of the early breeding programmes were not developed in accordance with modern views of population genetics (Princée 1990). The most important goal was successful reproduction i.e. breeding numbers. Avoidance of inbreeding and/or maintenance of genetic variation were not taken into consideration, until the end of the 1970s (see for example Flesness 1977).

Regional zoo associations established captive (or ex situ) breeding programmes in the early 1980s for zoo populations. The major goal of these programmes is to maintain viable populations without (or with limited) import from the wild. These managed zoo populations could also serve as potential resources for reintroduction and re-stocking programmes whenever feasible.

European zoos, associated in the European Association of Zoos and Aquaria (EAZA), have established European Endangered species Programmes (EEPs) and European Studbooks (ESBs) for some 250 species as at March 2002. The following sections will describe the various issues, from theoretical considerations to practical implementations, which play important roles in the daily practice of modern zoo breeding programmes.

Importance of genetic variation

Populations with low genetic variation are expected, according to evolutionary theory, to have lower adaptive potentials to cope with environ-

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mental changes than populations with high levels of genetic variation. The environment not only changes over time due to, for example, climatic changes but also fluctuates annually, seasonally and even daily. Therefore, genetic variation in a population is important to adapt (or to be adapted) to both short-term fluctuations and long-term changes in the environment. A classical example which illustrates this importance is the survival of dark forms of the peppered moth (*Biston betularia*) in industrialised (polluted) areas (Kettlewell 1973).

Zoo animals live in a relatively stable environment which prevents exposure to the harsh and extreme conditions, such as depletion of food resources, that may occur in their natural habitat. However, this does not imply that maintenance of genetic variation is not important for zoo populations. First, zoo populations could serve as resources for reintroduction. It is clear that a reintroduced population requires adaptive potentials to cope with the natural environment. Thus the source (=zoo) population must also have this adaptive potential. Second, zoo populations are also exposed to changes in environmental conditions, such as exposure to new viral strains that are transmitted by live-stock. Therefore, an important objective of zoo breeding programmes is to preserve at least 90 percent of the original 'wild' genetic variation for a period of 100 to 200 years (Soulé et al. 1986).

Genetic loss

Small populations lose genetic variation due to genetic drift. The smaller the population the larger genetic drift is (see Wright 1931). This can be illustrated with historical trends in population size and genetic variation in the zoo population of Przewalski's horses since 1900. Figure 1 shows population size, as computed from studbook data, and expected genetic variation as estimated with the genetic simulation ChromoFlow (Princée 1998). Two major historic trends can be observed. The first trend refers to a period of slow growth from 24 horses in 1901 to 58 in 1959. The second trend refers to the period 1960-1994 during which the population increased to 1293 horses. Gene diversity and observed heterozygosity declined until the mid 1960s. Note that gene diversity decreases gradually, while the decline pattern of observed heterozygosity shows fluctuations. Although gene diversity and observed heterozygosity have increased since 1965, both values as of 31 December 1994 were 84.5 and 79.6 percent of the original (wild) genetic variation, respectively.

Inbreeding depression

Inbreeding, i.e. mating between relatives, results in an increase in homozygosity in populations. This may result in reduced 'fitness', the relative ability of individuals to pass on gametes to the next generation. Individuals with a high proportion of homozygous loci often have lower fitness than heterozygotes, and deleterious recessive alleles can be expressed due to consanguineous matings of homozygotes. The effects of inbreeding on fitness are referred to as 'inbreeding depression' (Roughgarden 1979). Inbreeding depression resulting from the expression of lethal alleles or obviously detrimental alleles is more likely to be detected than that due to alleles that only slightly reduce fitness. For example, blindness in wolves (*Canis lupus*) has a genetic basis and only occurs in inbred packs descended from specific founder lineages in the Scandinavian zoo population (Laikre & Ryman 1991).

Figure 2 illustrates the probability (risk) that deleterious alleles are expressed in offspring of a full-sib (brother x sister) mating. Individual 5 has a probability of 0.25 (25%) that it is homozygous at a locus for one of the ancestral alleles of individuals 1 and 2. If these ancestors are carriers of deleterious alleles at multiple loci (genes) then the probability increases. Ralls et al. (1988) observed a range of lethal equivalents for survivorship of offspring in first-degree matings between -1.4 and 30.3 (a median value of 3.1) in 40 captive mammalian populations. Lethal equivalents can refer to single alleles at different loci or to combined lethal effects of several deleterious alleles at different loci.

Detecting inbreeding depression

Sensitivity to inbreeding depression may vary with species or even with populations (Shields 1987). Comparative studies between groups of inbred and non-inbred individuals, using quantitative measures that reflect fitness, are required to detect slightly deleterious alleles. Ralls & Ballou (1983) compared infant mortality between groups of inbred and non-inbred mammals living in zoos. This study showed that infant mortality in inbred groups was generally higher than in non-inbred groups.

Differences in sensitivity to inbreeding depression between species or populations, as observed in the study of Ralls et al. (1988), may be the result of past natural inbreeding in these species and populations. For example, natural populations that frequently undergo (and survive) severe bottlenecks are expected to be less sensitive to inbreeding depression than large outbred populations. Frequencies of recessive deleterious alleles in such populations are thought to be reduced in subsequent generations by both the bottleneck and inbreeding, which expose recessive alleles to selective forces.

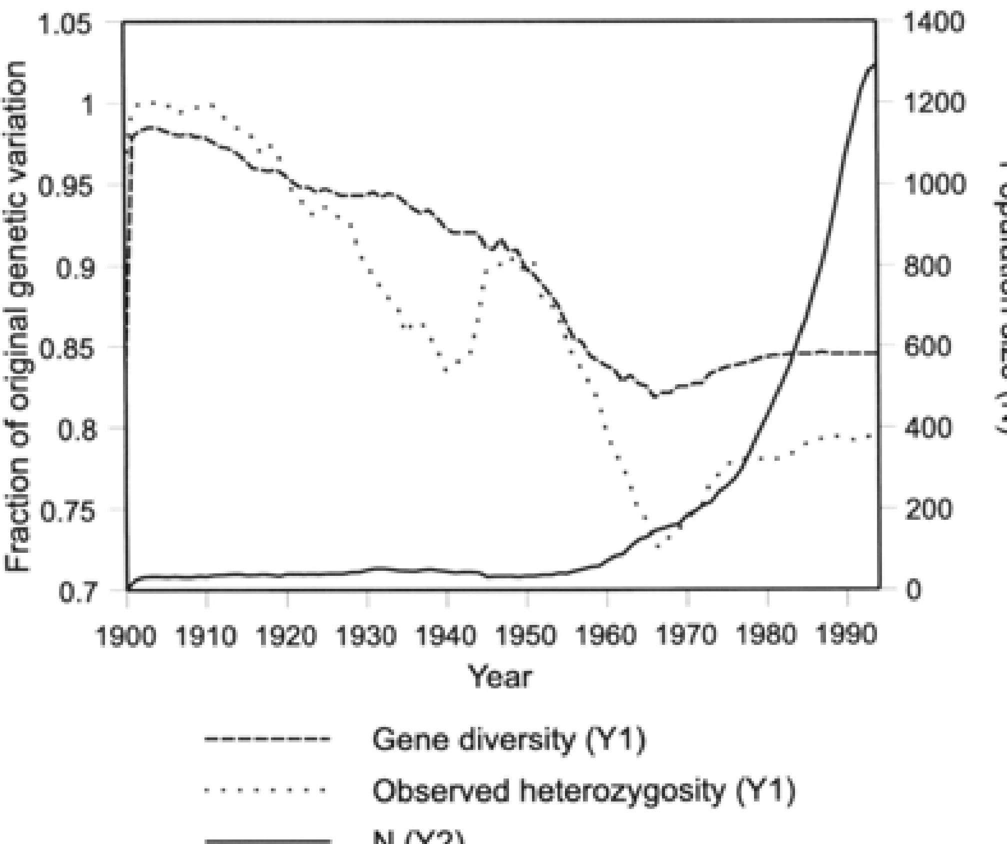
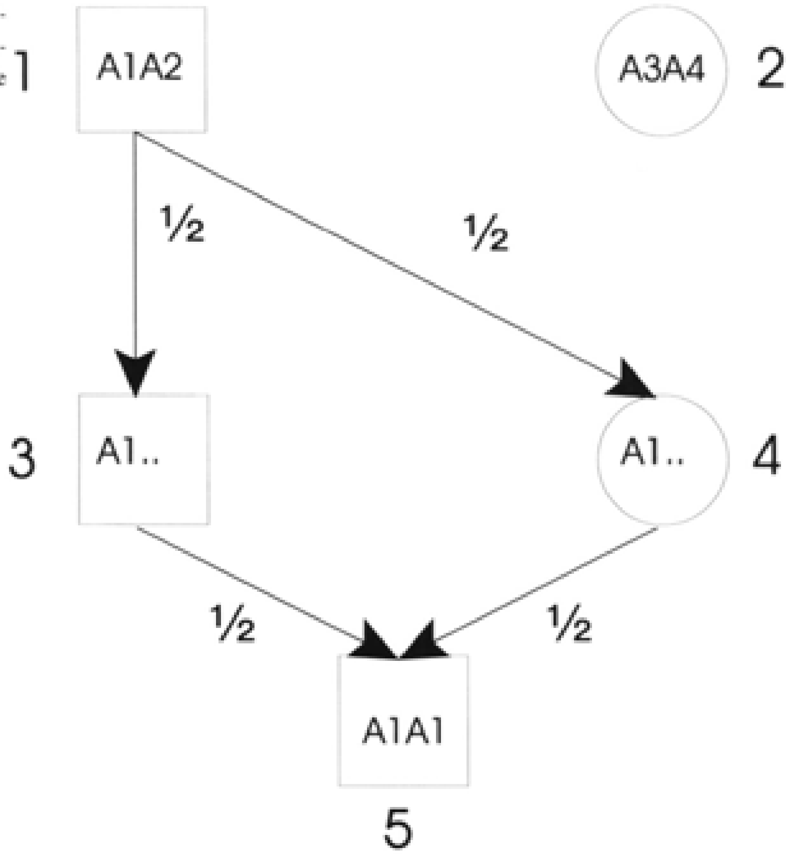


Figure 1. Numbers of living individuals (N; Y2-axis) and fractions of original (wild) gene diversity (Y1-axis) and observed heterozygosity (Y1-axis) as expected in the studbook population of Przewalski's horses (*Equus przewalskii*) at census dates (31 December) between 1900 and 1994. From: Princée 1998.

Figure 2. Pedigree of full-sib mating. See text for explanation. From: Princée 1998.



Inbreeding in natural populations

Inbreeding depression is not an artefact of zoo conditions, as suggested by some authors (e.g. Merola 1994), but has also been documented in various natural populations (e.g. Frankham 1995). A high incidence of malocclusion, an anomaly which results in shortened mandibles, has been observed in an isolated population of the red fox (*Vulpes vulpes*) in the North Holland Dune Reserve, The Netherlands. This population descends from a few founders and genealogical studies suggest that this anomaly has a genetic basis (Bouwmeester et al. 1989).

Sex-biased dispersal patterns of sub-adult or young individuals have been observed in various mammalian species like primates (Pusey & Packer 1987). Such dispersal patterns can be effective in avoiding full-sib matings, but do not fully exclude inbreeding. In this, the spatial distribution of social groups and the dispersal distances determine to what extent the level of inbreeding increases over generations in the population(s).

Studbook management

It is clear that maintenance of genetic variation and avoidance of inbreeding are important in order to establish self-sustaining zoo populations and to maintain the potential for future reintroduction. This genetic management requires that pedigree data of each individual are available to species managers. Data on individual animals in EEPs are maintained in studbooks. The International Species Information System (ISIS) has developed software - the Single Population Animal

Record Keeping System (SPARKS) - to computerise studbooks (Scobie & Flesness 1989). This software produces various reports such as historical listings, listings on birth and deaths, and reports per individual.

Furthermore, various additional computer tools have been developed to assist species managers in decision-making. For example, the programme GENES (Lacy 1994) estimates the expected level of genetic variation that has been retained in (zoo) populations, provides ranked lists of individuals that are considered genetically important, and also produces tables with inbreeding coefficients of possible matings.

Species managers can base breeding recommendations on the results of these genetic analyses. In this, logistics and (social) compatibility between potential breeding partners need also to be taken into account. For example, exchanges of animals between zoos in different countries (or continents), which require long periods of quarantine, may be less favoured.

Incomplete pedigree data

Genetic management of zoo populations is generally based on 'animal-by-animal recommendations' (Ballou & Lacy 1995), using tools as described above. However, this type of management is not applicable when pedigree data are incomplete. Information on parentages is often missing in populations of species with complex mating structures and/or species that are housed in colonies. However, genetic management of such species is not impossible. Princée (1995, 1998) developed 'low-intensity management' for social breeding groups. This type of management takes into account species-specific social (mating) structure and reproductive parameters such as gestation length, interbirth interval and reproductive life-span. These data can be used to reconstruct incomplete pedigrees.

Table 1 presents possible parents, based on biological data, of Przewalski's horses with unknown parents, which were born in herds in Askania Nova. This information can be used to reconstruct the gap in the pedigree of this zoo

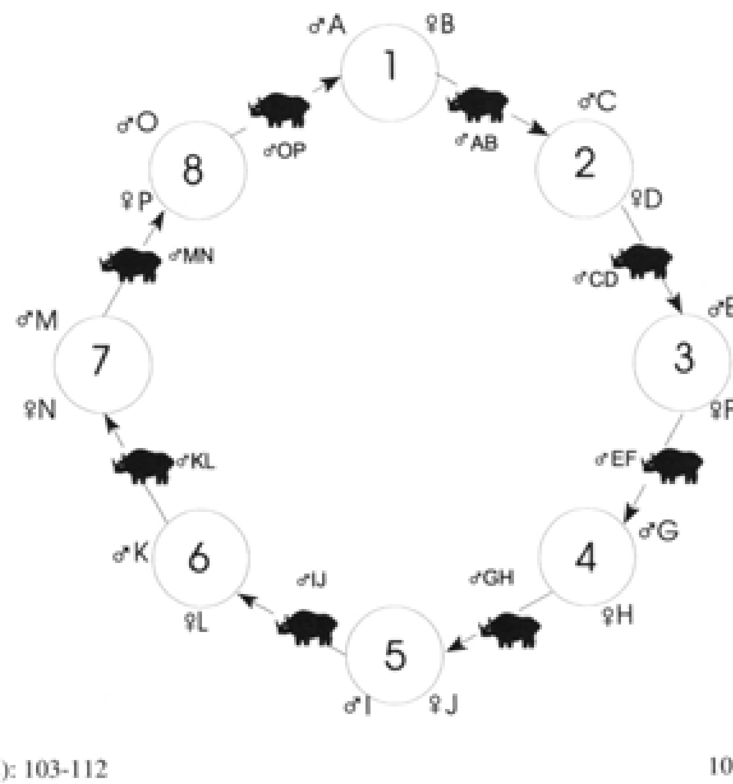
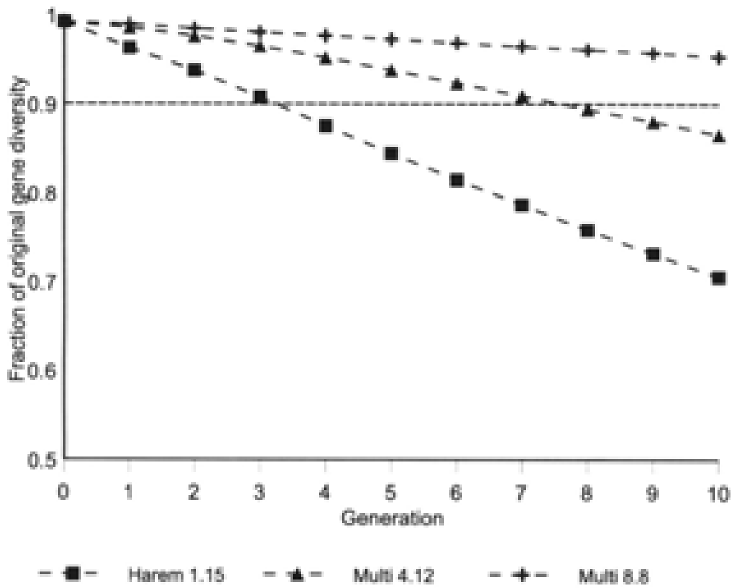


Figure 3. Maximal Avoidance of Inbreeding (MAI) scheme for eight breeding groups in the first generation. Bloodlines of paternal and maternal lines in each breeding group and bloodlines of male offspring are presented for each breeding group. Arrows indicate the dispersal of male offspring from natal to host group. From: Princée 1998.

Figure 4. Effect of number of breeding males in breeding groups on genetic loss. The number of males and females in groups are notated as M.F. (e.g. Harem 1.15). Indicated is the minimum fraction (0.9) of original gene diversity that should be conserved in small populations. From: Princée 1998.



population. However, reconstruction of pedigrees largely depends on the proportion of incomplete data. It may be obvious that this method cannot be used if too many assumptions have to be made.

Social groups

Low-intensity management can also be used to manage colonies as 'black-boxes'. Inbreeding in colonies can be avoided by exchanging animals according the Maximal Avoidance of Inbreeding (MAI) schemes (Wright 1921, Princée 1986, Princée 1995, Princée 1998). In this, information on natural dispersal patterns is used to select animals that will be exchanged between social groups. The MAI scheme uses single maternal and paternal lineages (or bloodlines) for each group. It assumes that all offspring are full-sibs, regardless of the actual composition of the breeding group. The MAI schemes are developed to avoid/minimise inbreeding in populations composed of social groups or colonies. Figure 3 shows a MAI scheme for eight breeding groups in the first generation. Princée (1998) developed algorithms to compute the exchange pattern for each group and for each future generation.

Since MAI schemes do not require information on pedigree patterns within groups or colonies, and exchanges are based on (sex-biased) dispersal patterns, they could potentially be applied in management of fragmented wild populations.

Genetic costs

Genetic variation is optimally preserved if all animals in a population produce equal numbers of offspring. However, the reality - both in captivity and in the wild - is different as most animals, especially those living in social groups with rank hierarchies, do not have the same chance to reproduce. This results in higher rates of genetic loss than under genetically optimal conditions. Princée (1995) used the simulation model GeneFlow to study the effects of social (mating) structure on genetic loss. Figure 4 shows genetic loss in populations of equal size (N=64) in which the number of breeding males per group is varied. Note that genetic loss in harem groups is substantially higher than in a population with groups that are composed of equal number of breeding males and breeding females.

Management measures

Management measures to reduce genetic loss in zoo populations focus on improving chances of reproductive success of individuals. In this, it would be tempting to house animals in breeding pairs (as often happened in the past) since this increases the chance of equal reproductive success. However, such a measure can dramatically violate the species-specific social structure, actually resulting in low reproductive success. An alternative measure is to maintain animals in the minimal group size that has been observed in natural populations.

Figure 5 shows results of simulations with the model GeneFlow (Princée 1998) on the effect of sizes of harem groups. Populations that are divided into small harem groups lose less genetic variation than populations with large harem groups. This means that genetic loss in social species can be reduced while the species-specific social structure can be maintained.

Wildlife management

Zoological gardens have almost 25 years of experience in population management that is

Long-term strategies: Przewalski's horses have been reintroduced in the original habitat in Mongolia almost a century after a breeding programme was initiated in zoological gardens. *Photograph: Edgar van der Grift.*

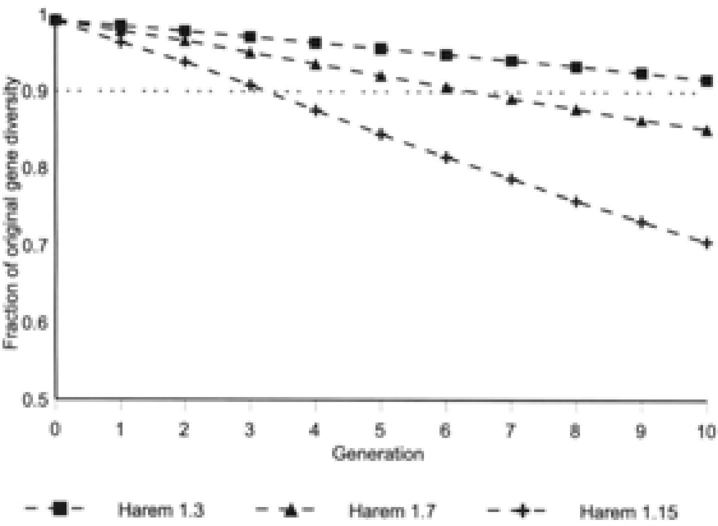


Figure 5. Effect of sizes of harem groups on genetic loss. The number of males and females in harem groups are notated as M.F. (e.g. Harem 1.3). Indicated is the minimum fraction (0.9) of original gene diversity that should be conserved in small populations. From: Princée 1998.

aimed at maintenance of genetic variation and avoidance (minimising) of inbreeding. In this, the introduction of the personal computer in the early 1980s has resulted in a rapid development of computer tools – often designed and programmed by zoo staff – to register data on zoo animals and to analyse genetic and demographic processes in zoo populations. In particular, the simulation models which are used to analyse pedigrees have generated more knowledge on the process of genetic loss in small populations (and the effects of social mating systems).

The experiences and knowledge of zoos are becoming increasingly important in management of wild populations. Habitat destruction and expansion of human activities result in further fragmentation and/or isolation of natural populations of endangered species. It means that animals may need to be translocated in order to avoid/minimise inbreeding in small sub-populations. In this, the MAI schemes could be applied in wildlife management to manage sub-populations in different nature reserves.

Acknowledgements: I would like to thank Dr. Anna Feistner (Durrell Wildlife Conservation Trust, Jersey) for critically reading this manuscript.

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Table 1. Possible parents of Przewalski's horses (*Equus przewalskii*) with unknown parents at Askania Nova. Numbers of individuals, dams and sires refer to international studbook numbers. Sire and dams that are presented in *italicised* text are assumed to be the parents. n/a = not applicable: sire or dam are known. From: Princée (1998).

#	Date of birth	Possible sire(s)	Possible dam(s)
826	22-05-79	n/a	*283*, 490, 528, 637, 675, 706, 711
966	12-05-81	*259*, 605, 764, 821	*283*, 525, 602, 620
1203	19-04-84	*259*, 533, 821, 893, 896, 900, 963, 970, 980, 1008	n/a
1214	04-05-84	*259*, 533, 821, 893, 896, 900, 963, 970, 980, 1008, 1044, 1048	n/a
1336	16-05-85	*259*, 533, 821, 893, 896, 963, 980, 1008, 1044, 1048	244[1] , *490*, 1077, 1114
1339	19-05-85	n/a	244[1] , 490, *1077*, 1114
1478	13-06-86	n/a	490, *528*, 620, 959, 987, 1114, 1215, 1221
1550	02-05-87	n/a	*987*, 1043, 1215, 1221
1553	04-05-87	n/a	987, *1043*, 1215, 1221
1598	05-06-87	*533*, 821, 893, 896, 963, 980, 1008, 1044, 1048, 1145, 1178, 1214, 1231, 1331, 1338, 1348	987, 1043, *1215*, 1221
1637	08-08-87	*533*, 821, 893, 896, 963, 980, 1008, 1044, 1145, 1178, 1214, 1231, 1331, 1338, 1348	n/a
1724	30-05-88	*533*, 821, 893, 896, 963, 980, 1008, 1044, 1145, 1178, 1214, 1231, 1331, 1338, 1348	*525*, 528, 812, 846 1077, 1215, 1456, 1464, 1467, 1468
1754	26-06-88	*533*, 821, 893, 896, 963, 980, 1008, 1044, 1145, 1178, 1214, 1231, 1331, 1338, 1348	525, *528*, 812, 846, 1077, 1215, 1456, 1464, 1467, 1468, 1478
4035	18-04-89[2]	n/a	812, 987, *1077*[3], 1456, 1468, 1478, 1511
4733	29-11-89[2]	*533*, 821, 893, 896, 963, 980, 1008, 1044, 1145, 1178, 1214, 1231, 1331, 1338, 1348, 1500, 1568, 1598, 1607	812, 1456, *1467*[4], 1468, 1478, 1595, 1600
4502	05-05-90	n/a	*525*, 528, 812, 846, 1456, 1595, 1703
4657	18-05-90	*1619*	*1639*
4056	03-11-90[2]	n/a	*1305*
4077	15-02-91[2]	n/a	*1265*, 1276
4064	16-09-91[2]	n/a	*859*
4072	22-08-92[2]	n/a	*859*
5070	02-09-92	*1720*	n/a
5097	11-05-94	*1178*, 1331, 1348, 1697, 1920, 1976	n/a

[1] Dam 490 was beyond reproductive age (and died in June 1986)
[2] Abortion or stillbirth
[3] Died at same date as date of birth of 4035
[4] Produced 4038 (stillbirth) at same date as date of birth of 4733
Source: International studbook of Przewalski's horses

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Samenvatting

Genetisch beheer van kleine dierpopulaties

Europese dierentuinen die lid zijn van de European Association of Zoos and Aquaria (EAZA) startten in 1985 met gecoördineerde fokprogramma's voor dierentuin populaties. Ruim 250 European Endangered species Programmes (EEPs) en Europese studbooks (ESBs) zijn sinds die tijd opgezet. Het doel van fokprogramma's is het behouden van levensvatbare dierentuinpopulaties zonder (of met sporadische) import van wildvang-dieren. Deze populaties dienen, wanneer mogelijk, als bronnen voor herintroductie programma's van bedreigde diersoorten.

Behoud van genetische variatie in populaties van bedreigde diersoorten wordt als een belangrijke voorwaarde voor het overleven beschouwd. Dit betekent dat genetisch beheer van dierentuinpopulaties, zeker als herintroductie een rol speelt, in behoudsprogramma's moeten zijn opgenomen. Populatiebiologen die werkzaam zijn voor dierentuinen hebben verschillende strategiemodellen en computerprogramma's ontwikkeld ten behoeve van het gezamenlijk beheer van dierentuinpopulaties.

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Genetic management of European otters (*Lutra lutra*) in European zoos

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Abstract: The European otter (*Lutra lutra*) is considered an endangered species in Europe. Therefore European zoos started a co-operative breeding programme – a so called European Endangered species Programme (EEP) - for this species in 1990. An important goal of the European otter EEP is to maintain an *ex-situ* (i.e. captive) population without imports of animals from the wild. Moreover, this population will be an important resource for reintroduction projects for this species. A reintroduction project of European otters in The Netherlands started in July 2002. Genetic management to avoid inbreeding and to maintain genetic variation is a pre-requisite to maintain a viable population. Examples of management strategies are described in this paper.

Keywords: Lutra lutra, European otter, captivity, EEP, genetics, management, zoo.

Introduction

The European otter (*Lutra lutra*) is considered to be an endangered species in Europe. Although healthy populations still remain in several East European countries, Portugal, and Great Britain, this species went extinct in most of the continental West European countries. The threats are numerous and differ per country or region, but in general the main causes for the disappearance of the otter from our wetlands and rivers are the loss of suitable habitat, fish traps, traffic casualties and bio-accumulation of toxic agents in the food chain causing decreased fertility and increased susceptibility to disease.

The European otter has been adopted as the ambassador for freshwater habitats by several countries in recent years. These countries aim to conserve and restore sufficient areas, linked with corridors, to maintain viable otter populations in the wild. Several reintroduction projects have been established in Sweden, Spain and Great Britain, and other countries have planned releases within the next few years (France, Italy). Reintroduction of seven European otters in The Netherlands took place in July 2002. A second group of eight animals was released in October 2002. Reintroduction involves both translocation of otters from other wild populations and releases of captive-born animals.

Guidelines for reintroduction require that animals which are to be introduced should be as closely related genetically with the original native population as possible (IUCN 1995). This means that the origin of each of these animals should be known. Information on the capture site will be sufficient for animals which will be translocated. Data on parentages in order to trace origins of the founders are required for zoo-born animals which will be released. Such data are stored in studbooks. An international studbook for European otters was established in 1986.

Captive animals to be used in reintroduction must be from populations which have been soundly managed both demographically and genetically, according to the principles of contemporary conservation biology (IUCN 1995). A co-operative population management programme, i.e. European Endangered species Programme (EEP), for European otters in European zoos was

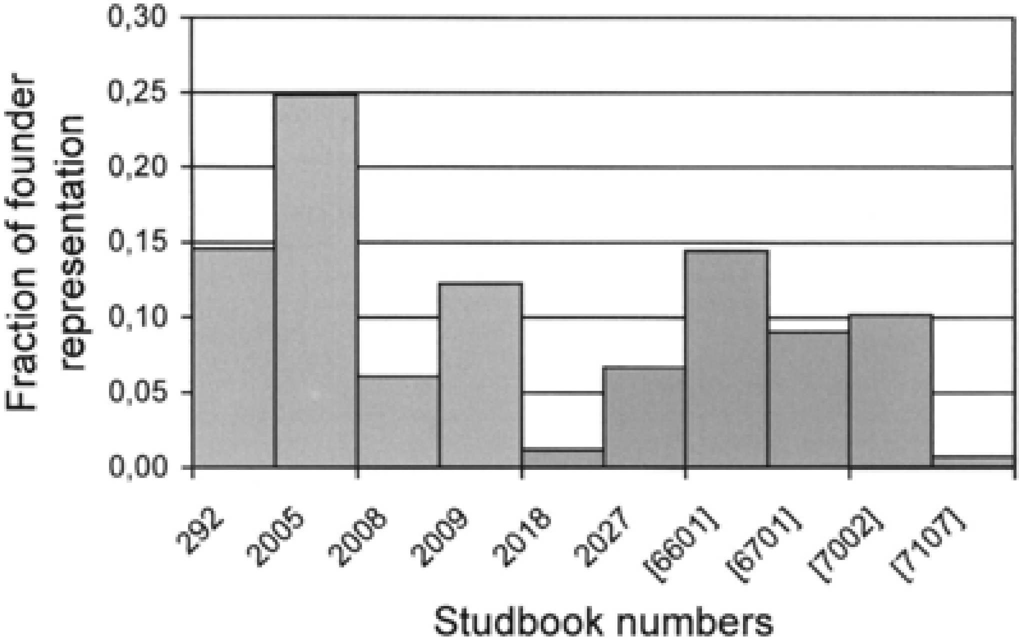

Figure 1. Founder representation in the Dutch zoo population of European otters (*Lutra lutra*) as on 1 January 2001. Studbook numbers between brackets refer to pseudo-founders.

established in 1990. The goals of this programme are to maintain a viable zoo population that does not depend on imports from the wild and that serves as a resource for reintroduction projects. For example, a reintroduction programme in The Netherlands will require 45 otters within the next five years. The first release of European otters in The Netherlands, in July 2002, involved two animals that were born in a Swedish zoo and are part of the EEP for this species. These otters are registered in the studbook under numbers #2676 and #2677.

EEP programmes involve the development of husbandry guidelines (containing the most current knowledge on housing, nutrition and breeding the species), initiation and coordination of research (for example on reproduction and diseases), and population management. Population management involves control over population size (demographic management) and avoidance of inbreeding and maintenance of genetic variation (genetic management). This paper will present some techniques that are applied in the genetic management of the zoo population of European otters, using the otter subset in Dutch zoos as an example.

Studbook data

Studbook data, including identification marks, parentages, dates of birth, transfers between zoos and dates of death, on individual animals are computerised in the program Single Species Animal Record Keeping System or SPARKS (Scobie & Flesness 1989). This programme allows the production of various overviews and listings, such as the history of the population or just the living population. The population of European otters in European zoos numbers 455 animals in some 80 institutions (data current as of January 2001).

The add-on programme GENES (Lacy 1994) is used for genetic analyses. GENES computes various genetic measures such as inbreeding coefficients and mean kinship values for each individual animal, and founder representation in (subsets of) the living population. These data are used to determine which animals should be paired and which animals should be allowed to breed.

Founder representation and mean kinship

Founder representation in a zoo population refers to the proportion of genes from wild-born animals that are present in the living captive (zoo-)born animals. Figure 1 shows the founder representation in the living population of European otters in Dutch zoos as on 1 January 2001. The history of breeding success with this species in zoos is reflected in an unequal founder representation. Only a few zoos amongst those originally holding them were successful in breeding from wild European otters (and later their offspring) and so the genes of these first founders are represented in higher proportions than the genes of other founders, which were added to the population later.

The overview of founder representation is used to determine which animals are important for breeding in order to equalise founder representation, when feasible. It is clear that it will be important to breed with wild-born animals which have either not reproduced or are under-represented in the population. However, it becomes more difficult to select genetically important individuals in populations with complex pedigrees (see for example Princée 1988), especially when such individuals are descendants from multiple founders which are over- and under-represented in the population. Because of this the genetic measure "mean kinship" is used to identify genetically important individuals in the population. Mean kinship refers to the average genetic relatedness of an individual to the other individuals in the population. A mean kinship of 0.00 refers to an individual which is unrelated to all others. Such an individual is either wild-born and has not reproduced or is the single living offspring of a founder (or founders), and being thus genetically important would be a priority for breeding. A mean kinship of 1.00 means that all individuals are genetically identical ("clones"). See Ballou & Lacy (1995) for further details.

Mean kinship values for the 10 genetically most important males and females in the Dutch zoo population are presented in table 1. This table shows that all European otters currently in Dutch zoos are related so it is therefore not possible to establish new breeding pairs within this subset of the European zoo population. This

Table 1. Ranked lists of mean kinship values (MK) for European otters (*Lutra lutra*) in the Dutch zoo population ordered by sex as on 1 January 2001. Stud# = Studbook number; MK= mean kinship value; Known = fraction of the genome which originates from known ancestors.

Rank	Males				Females			
	Stud#	MK	Age (yrs)	Known	Stud#	MK	Age (yrs)	Known
1	1306	0.1208	7	1.00	1334	0.1966	6	1.00
2	2131	0.1502	6	0.50	1284	0.2176	8	0.25
3	2132	0.2499	6	0.38	2133	0.2440	6	0.38
4	2176	0.2638	4	0.75	2335	0.2972	2	0.25
5	2450	0.2846	1	0.44	2336	0.2972	2	0.25
6	2451	0.2846	1	0.44	2579	0.3084	0	0.56
7	2340	0.2846	2	0.44	2580	0.3084	0	0.56
8	1351	0.2862	7	0.5	2175	0.3116	4	0.75
9	2234	0.2972	3	0.25	1152	-	13	0.00
10	2484	0.3084	1	0.56	1240	-	10	0.00

Guidelines for reintroduction of otters in the wild require that animals which are to be introduced should be as closely related genetically with the original native population as possible. *Photograph: Edgar van der Grift.*

means that further exchanges with zoos in other countries, following mean kinship tables for the living population in Europe, are required.

Unknown parents

Some institutions unfortunately had not maintained records of the animals in their collection when the European otter studbook started some 20 years ago. Therefore valuable information on animals in the studbook is not always available (or accurate). It is clear that unknown data for parentages directly affect the results of genetic analyses. The programme GENES can handle "unknown" genes i.e. genes from unknown founders (Lacy 1994). The programme adds pseudo-founders as parents for unknown sires and dams. Founders 6601, 6701, 7002 and 7107 in the founder representation in the Dutch zoo population are pseudo-founders (see figure 1). These pseudo-founders contribute to almost 35% of the genes in this population subset. This large percentage can be explained; these zoos received animals from the few successful breeding institutions in the past.

The unknown origin of genes in the genome of individuals is also reflected in the mean kinship list. For example only 25% of the genome of female 1284 can be traced to known founders (see table 1).

Future management

The genetic situation in the European zoo otter population as a whole does not differ much from that illustrated for the Dutch zoo population subset. Large numbers of animals are of unknown or of partly known origin. Therefore the European otter population is managed in two groups. The "A" group is composed of otters whose origin is completely known. These animals are allowed to breed. The A-line otters or their offspring can be used for reintroduction programmes, as recommended by the IUCN/SSC guidelines. This group currently comprises 30% of the living European zoo population.

The B-line otters are restricted in breeding and are currently maintained for conservation education display purposes only. The restricted breeding in this group should result in its complete replacement by animals of known genetic origin (i.e. the A-line) within the next 25 years.

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Samenvatting

Genetisch beheer van Europese otters (*Lutra lutra*) in Europese dierentuinen

De Euraziatische otter (*Lutra lutra*) is een bedreigde diersoort in Europa. Daarom zijn de verenigde Europese dierentuinen een speciaal fokprogramma begonnen waardoor de dierentuinenpopulatie zonder verdere wildvang in stand kan worden gehouden. Bovendien kunnen uit deze populatie op termijn dieren gebruikt worden voor diverse herintroductie-projecten in Europa. Methoden en technieken die worden gebruikt bij het genetisch beheer van dierentuinpopulaties worden in deze bijdrage besproken.

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