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Genetic and demographic management of conservation breeding programmes oriented towards reintroduction

Gestión genética y demográfica de los programas de cría en cautividad con fines de reintroducción

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Resumen

En el presente capítulo se resumen los principios generales de la gestión genética de los programas de cría en cautividad con fines de reintroducción, uno de cuyos objetivos más importantes es mantener tanta diversidad genética como sea posible. Esta diversidad genética representa el potencial evolutivo de una población y está correlacionada con su aptitud biológica. Las poblaciones en cautividad suelen ser pequeñas, carecen de flujo génico sin intervención humana y no viven en condiciones naturales. Esto las hace vulnerables a cambios genéticos que pueden afectar al éxito de la reintroducción, tales como la pérdida de diversidad genética debido a la deriva genética, la endogamia, la depresión por endogamia y la adaptación genética a la vida en cautividad. La mejor forma de mantener la diversidad genética en poblaciones cautivas implica: 1) maximizar el número de ejemplares fundadores (sin poner en peligro la población silvestre de donde se extraen los ejemplares), 2) maximizar la tasa de crecimiento durante la etapa de crecimiento de la población (lo que implica un buen conocimiento de la historia natural y los cuidados que requiere la especie en cautividad), 3) maximizar la capacidad de carga y 4) realizar los emparejamientos de los ejemplares en función de su índice medio de parentesco o mean kinship (mk), sobre todo durante la "etapa de capacidad" del programa, y además, 5) minimizar la endogamia. El valor mk de un individuo es una medida con la que se establece su parentesco con toda la población. Los ejemplares con valores de mk bajos tienen pocos parientes en la población. Si uno de estos individuos muere, es muy probable que se pierda para siempre esa diversidad genética única. En cambio, es probable que la mayor parte del material genético de un individuo con muchos parientes ya esté representado en ellos. Por tanto, la diversidad genética de una población cautiva

se puede maximizar dando prioridad a los individuos con valores de mk bajos, emparejando individuos con valores de mk similares para la reproducción, y minimizando la endogamia. Se necesita un buen grado de conocimiento para tener en cuenta las características biológicas y sociales de la especie, las peculiaridades no genéticas de los individuos en cuestión y las circunstancias prácticas. Por último, los individuos criados en cautividad más idóneos para la reintroducción deben estar bien representados en la población cautiva y, asimismo, deben ser cuidadosamente seleccionados, ya que ellos mejorarán la diversidad genética de la población silvestre. Todos estos principios se explican en este capítulo, prestando particular atención al caso específico del lince ibérico.

Palabras clave
Reproducción en cautividad, diversidad genética, fundadores, metas poblacionales, selección de parejas, índice medio de parentesco

Abstract
This paper aims to summarise the general principles of the genetic management of conservation breeding programmes with the aim of reintroduction. One of the most important aims for such programmes is to retain as much gene diversity as possible. Gene diversity represents the evolutionary potential captured within the population and is correlated with population fitness. Populations in captivity are often small, lack gene flow between subpopulations without human intervention, and live under unnatural conditions. This makes captive populations vulnerable to genetic changes that may affect reintroduction success, such as loss of genetic variation through genetic drift and inbreeding, inbreeding depression, and genetic adaptation to captivity. Gene diversity can best be maintained in captive populations by 1) maximising the number of founders (without compromising the wild source population); 2) maximising the growth rate in the growth stage of the population (implying good knowledge of natural history and captive husbandry); 3) maximising carrying capacity, and 4) basing the pairings of individuals, especially during the capacity stage of the Programme, on their mean kinship (mk) values, while 5) minimising inbreeding. The mk value of an individual is a measure for the relatedness of this individual to the entire population. Animals with low mk values have few relatives in the population and vice versa. If an individual with few relatives dies, chances are high that unique genetic variation is lost forever. In contrast, most of the genetic material of an individual with many family members (i.e., high mk) is likely also present in its relatives. By giving breeding priority to low mk individuals, combining individuals with similar mk values for mating, and minimising inbreeding, the amount of gene diversity retained can be maximised. A degree of compromise will be necessary to take into account the biological and social characteristics of the species, non-genetic peculiarities of the individuals involved, as well as practical circumstances. Finally, the captive born individuals best chosen for reintroduction are those that benefit the gene diversity of the wild population, but are genetically well represented in the captive population. The above principles are explained while paying particular attention to the specific case of the Iberian lynx.

Keywords
Captive propagation, gene diversity, founders, population targets, pair selection, mean kinship

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Introduction: Why is genetic management so important?

One of the most important aims for the management of any captive population of endangered species, and especially one that is to function as a source for individuals to be reintroduced into the wild, is to retain as much gene diversity as possible. This is because gene diversity represents the evolutionary potential captured within the population and because there is a correlation between gene diversity (heterozygosity) and population fitness (Reed and Frankham, 2003). The more variation in genetic traits present in the population and in the individuals within it, the higher the chance that at least a proportion of the individuals is able to survive the challenges offered by the variable wild environment. Populations in captivity are often small, lack gene flow between subpopulations (different enclosures or captive colonies) without human intervention and live under unnatural conditions (de Boer, 1989). These characteristics make captive populations vulnerable to a number of genetic changes that affect reintroduction success, among which loss of genetic variation (and therefore of evolutionary potential) through genetic drift and inbreeding, inbreeding depression, and genetic adaptation to captivity. Gene diversity can be measured by employing various molecular techniques to study the nuclear and/or mitochondrial DNA of a sufficiently large sample of individuals in the population, or can be inferred from detailed pedigree records with the aid of computer modelling.

If captive propagation is expensive and prone to losing gene diversity, why do it?

The maintenance of a captive population of an endangered species is a very costly affair. If captive populations are vulnerable to rapid loss of evolutionary potential, and at the same time cost a lot of time and resources, why do it? The same artificial conditions that cause the loss of gene diversity in captivity also provide the captive population with a relatively safe environment (no predators, sufficient food and shelter, medical treatment etc.). Assuming one has sufficient knowledge of the basic biology and therefore husbandry needs of the species, a much faster population growth can be obtained in captivity. Mortality rates may be lower in captivity than in the wild and for some species the reproductive output per female per year can be increased (e.g., double clutching in birds). The assumption of sufficient knowledge of the biology and husbandry of the species is not one to be taken lightly. If reproduction is lower and/or mortality is higher in captivity than in the wild, extracting animals from the wild to establish and support the captive population will act as a drain on the already endangered wild population. Sometimes closely related species for which there is more knowledge on the natural history in the

wild and/or that have already been kept successfully in captivity can act as a model for the development of suitable husbandry and breeding techniques. In the case of the Iberian lynx for example, experience with the Eurasian lynx *(Lynx lynx)* taught that in captivity there is regular mortality of cubs due to one cub attacking and fatally injuring another cub of the same litter. This knowledge allowed housing and breeding management in the Iberian lynx *ex situ* breeding programme to be adapted such that this cause of cub mortality could be minimised without compromising the family unit and normal lynx rearing behaviour (Vargas et al., 2005).

Apart from providing a safe environment, the more controlled conditions in captivity allow for pro-active genetic and demographic management in order to minimise and slow the rate of loss of gene diversity, even when the population is relatively small. The relevant techniques employed will be discussed below.

Starting the captive population – how many founders?

The wild caught individuals at the basis of a captive population are known as founders. The founders are a sample of the wild population and therefore the statistical laws of sampling apply (Lacy, 1994). The larger the number of founders taken from the wild population, the higher the proportion of the wild population's gene diversity that will be present in the source population. In the case of critically endangered species, this creates an interesting dilemma. Catching many wild individuals as founders for the captive population may further endanger the wild source population(s). Also, the smaller and more fragmented the wild population becomes, the less gene diversity is available for the start of a captive population. For this reason IUCN recommends in its guidelines for the *ex situ* management of endangered species, to start a captive breeding programme when a species still numbers in the thousands in the wild (IUCN, 1995). Considering the very low numbers of Iberian lynx in the wild, there was no time to lose and the decision to start an *ex situ* breeding population was entirely warranted (Delibes et al., 2000).

If catching more founders is better for the captive population but less good for the remnant wild population, then how many founders are enough? Luckily, sampling and genetic theory indicates that only modest numbers of founders, namely at least 20 unrelated wild individuals, are sufficient to capture 97.5% of the gene diversity of the wild population within the founder population. Even a modest further increase in amount of gene diversity captured would require many more founders (Crow and Kimura, 1970; de Boer, 1989; Lacy, 1994; Frankham et al., 2002). About 20 unrelated founders are therefore considered an acceptable compromise between gene diversity captured and number of individuals needing to be extracted from the endangered wild population.

At this stage it is worth pointing out a few caveats: 1) A wild caught-individual can only be truly considered a founder once it has surviving descendants in the captive population. Therefore, likely more than 20 individuals will have to be caught as not all individuals may breed. 2) The founder generation is only the start of the captive breeding programme. How breeding progresses from the founder generation onwards (determined by management and the natural history characteristics of the species which influence growth rate, population size, founder representation, inbreeding level, etc.) will determine the rate of loss of the gene diversity present in the founder population. Twenty wild caught individuals should therefore be considered a theoretical minimum (see further in this paper for what is appropriate specifically for the Iberian Lynx population).

Breeding from the founders – more is better!

Each generation of individuals born in captivity is in a genetic sense a sample of the previous generation (Lacy, 1994). This can be understood as follows (de Boer, 1993): Every time a parent has an offspring, 50% of its genetic material is passed on to that offspring. If a parent has a second offspring and if the transmittance of the genetic material to a second offspring is independent of that to the first one, it is highly unlikely that they will each receive either exactly the same 50%, or the complementary 50%. On average, 50% of the genetic material will only be present in one offspring and not in the other, 25% will be present in both and 25% in neither. Statistically speaking, the genetic material of the two offspring combined represents 75% of the genetic material of the parents. If the parents have three offspring, this becomes 87.5%, four 93.75% etc. Conversely, should the parents die after having had three offspring, on average 12.5% of their genetic material would not be represented in those offspring – therefore 12.5% of their genetic variation would be lost. All of this holds a number of implications:

1. The more offspring a founder produces, the more of its genetic variation will have been passed on to the

next generation. In real life, the above theory holds true for each founder allele separately and some loci will be linked on the same chromosomes and are therefore not transmitted independently. Things are therefore not quite that simple, but it has nevertheless been estimated that 12 offspring per founder are sufficient to provide 99% probability that all alleles of a founder are transmitted to at least one offspring (Thompson, 1994).

2. The offspring will only be able to retain and preserve the genetic variation of the founders if they themselves survive and reproduce before they die. Genetically speaking, a founder that has had 12 offspring, but of which 9 never bred, might from a genetic point of view as well only have had three offspring. Therefore, it is likely that more offspring need to be produced than sampling theory indicates (to compensate for stochasticity in survival and reproduction of the offspring).

3. As each founder needs to produce many offspring, a steep growth rate is an important goal in the foundation phase of a captive breeding programme.

4. In captive populations that are small and remain small, a lot of gene diversity will be lost quickly due to the above process. Which alleles are lost is largely due to chance (a process called genetic drift), especially if there is no proactive management of who will breed with whom in the capacity phase of the population (see below).

5. Gene diversity is lost per generation. Therefore the fewer generations a population passes through in a given number of years, the slower gene diversity will be lost. This is partly determined by the natural history of a species, but can also be influenced to some degree by breeding management.

6. The growth rate during the foundation phase strongly influences the rate at which gene diversity is lost in the future of the captive breeding programme. In addition, when a founder dies, any of its genetic variation that was not yet passed on to the next generation will have been irretrievably lost from the captive population. There is also always a chance that mortality may occur among the offspring of a founder before they themselves have had the chance to reproduce and pass on the retained gene diversity. For these reasons, as long as carrying capacity in captivity has not yet been reached, founders should not be prevented from breeding, even those founders that appear to be much more prolific than the others. It is preferable to try to correct unevenness in founder representation during the capacity stage of the programme, rather than to risk losing alleles during the founding stage (Lacy, 1994). As long as all founder alleles are passed on with an acceptable probability, the fact that some have a higher frequency than others can be addressed later.

Capacity stage – how many Iberian lynx are enough?

From a genetic point of view, the smaller a population at the carrying capacity stage of a breeding programme (the stage at which the population will be kept stable at that size), the more gene diversity is lost. Although bigger is better, both space and financial and human resources are always limited. This begs the question, how big is big enough? What should be the minimum population of Iberian lynx kept in captivity in order to be able to retain a sufficient amount of gene diversity? And how much gene diversity is enough? This leads to the search for an acceptable compromise between the theoretic ideal and what is possible in practice.

First, it is necessary to introduce the concept of Effective Population Size (Ne). This theoretical concept can be intuitively understood by taking into consideration that a population with 500 males and 20 females is not as "effective" as one with 260 males and 260 females, or that having only 10% of the males doing all the breeding is not genetically the same as having all reproductive aged males contributing equally to the next generation. Or that a population with 500 individuals that crashes to 50 and then grows back to 500, is not as "effective" as one that had a stable population size of 500.

The effective population size is defined as the size of an ideal population that would have the same rate of genetic drift and of inbreeding as is observed in the real population with N individuals (Lacy, 1994; Frankham et al., 2002). In an ideal population there is random breeding, constant population size, equal sex ratio and non-overlapping generations. Needless to say, real life populations are far from "ideal". The ratio of Ne to N is influenced by the

number of breeding animals in the population (some are pre- or post-reproductive and some animals at reproductive age may not breed for other reasons), variation in family size (not all individuals produce the same number of offspring), unequal sex ratio (leaving some animals of the more abundant sex with fewer breeding opportunities), and fluctuations in population size. There are different methods of calculating Ne that each makes adjustments for these different parameters influencing Ne (Frankham et al., 2002).

There is a relationship between the effective population size and the gene diversity retained: $Gt=G0\ (1–1/(2Ne))^t$, whereby Gt is gene diversity retained at generation t, G0 is gene diversity present in generation 0 (Frankham et al., 2002). The higher the effective population size, the more gene diversity will be retained. This implies that, apart from the number of founders, the growth rate and the true size of a population, the amount of gene diversity that can be retained in a captive population also depends on how closely the true population behaves to the ideal population. No real population will achieve the theoretical 'ideal', but a technique for management of breeding in captive populations has been developed to help maximise the ratio of Ne/N in captive populations (see below).

Year	N	Capture of founders	Releases	Cumulative releases
2004	6	0	0	0
2005	11	4 + 1	0	0
2006	18	4	0	0
2007	25	4 + 1	0	0
2008	35	4	0	0
2009	46	4 + 1	0	0
2010	56		5	5
2011	67	1	5	10
2012	73		8	18
2013	72	1	13	31
2014	73		12	43
2015	73	1	13	56
2016	72		12	68
2017	73	1	13	81
2018	73		12	93
2019	72	1	13	106

Figure 1. Growth projections for the Iberian lynx Conservation Breeding Programme (from Lacy and Vargas, 2004).

Figura 1. Proyecciones de crecimiento del Programa de Cría para la Conservación del Lince Ibérico (Lacy y Vargas, 2004).

Current genetic theory indicates that the minimum viable population size needed to balance the loss of gene diversity due to drift with the generation of new diversity through mutations is an effective population (Ne) of 500 - 5.000, which for wild populations often corresponds to a true population size (N) of about 5.000 – 50.000 individuals (Thomas, 1990; Nunney & Campbell, 1993; Frankham et al., 2002). Even when taking into consideration that the Ne/N ratio for captive breeding programmes under proper genetic and demographic management is often close to 0.3 (Frankham et al., 2002; Mace, 1986), this still implies a required true population size of about 1.700 – 17.000 which is a practical impossibility in terms of space, finances and resources for the vast majority of programmes. Even when space for a captive breeding programme is shared between a large number of zoos and other holding collections, the number of species needing captive breeding is so high that the demand for space usually far exceeds what is available. However, if a modest amount of loss of gene diversity is accepted, a smaller population is required to achieve this goal. Currently, the world zoo and aquarium community generally considers a goal of retaining 90% of gene diversity present in the source population after 100 years of breeding in captivity to be an acceptable compromise between a modest loss of gene diversity and accommodating more breeding programmes (because they are of smaller size). This goal can generally be achieved with a few hundred, rather than a few thousand individuals. Ninety percent of gene diversity retained after 100 years corresponds to an average level of inbreeding of 10%, meaning that on average the individuals would be related to the equivalent level of just below that of half-siblings or that of aunt and nephew (F = 0.125).

How has all this been applied to the particular situation of the Iberian lynx? The software programme PM2000 was designed to establish the genetic and demographic goals for pedigree managed captive breeding programmes (http://www.vortex9.org/pm2000.html) (Pollak et al., 2007). Based on the generation length, the population growth rate, the current population size, the current effective population size, the ratio of Ne/N, and the current gene diversity, me calculates the population size needed to reach a particular genetic goal (e.g., retention of 90% of gene diversity at the end of 100 years). These population parameters can either be calculated from pedigree data, or can be entered by the user. Lacy and Vargas (2004) employed PM2000 in order to determine

the goals for the captive Iberian lynx population. At the time of the analyses, the *ex situ* Iberian lynx population consisted of a total of six animals, all wild caught animals. As no studbook data were available that could be used to calculate the other parameters, a number of assumptions were made, based on experience with similar species: maximum annual population growth rate 21.5%, generation time 5.25 years, Ne/N ratio 0.3, and current gene diversity 90%. The analyses indicated that the goal of maintaining 90% of gene diversity for 100 years is not obtainable for the Iberian lynx because the number of extra founders needed to achieve this (12 extra founders per year for the next 5 years) is more than the wild populations can sustain (Palomares et al., 2002), and the number of individuals required at carrying capacity (500) is exceeding the availability of space and resources for the programme. Furthermore, the primary goal of this captive population is not to provide a long term (e.g. 100 years), large, back up population for the wild population, but to provide a genetically healthy, yet relatively small, short term population that can relatively quickly supply individuals for reintroduction. Further modelling indicated that it will be possible to maintain 85% of gene diversity for 30 years (a more realistic time span) with a nucleus population of 60 breeders (feasible in terms of space and resources), if 4 wild cubs can be added to the programme each year, for the next five years (spread over Doñana and Sierra de Morena – a rate deemed viable according to the study by Palomares et al., 2002), as well as one extra founder every two years for the whole duration of the programme in the form of animals entering rescue centres (Figure 1).

Is 85% gene diversity retained (and therefore an average level of inbreeding after 30 years of 15%) much worse than 90% (and 10% inbreeding) and will this compromise the success of reintroduction? This is again a matter of probabilities. There is no guarantee that a captive population, or a reintroduced population derived there from, with less gene diversity and higher inbreeding levels will go extinct or suffer in other ways, but the chance that it does increases. It is therefore always a case of trying to achieve the highest possible retention of gene diversity within the restraints of the particular situation of the species and the space and resources available.

Who breeds with whom?

From a genetic point of view, what you would ideally like to do in an *ex situ* programme is magically freeze the founders and thaw them out again at some point in the future so they can be available for breeding. In that way, all gene diversity would have been retained and all allele frequencies would remain exactly the same. Although gene banking and reproductive technologies allow us to take important steps in this direction (Ballou, 1984), generally the gene diversity of the founders is preserved for the future by having them breed by natural means and pass on their genes to the next generation. Ideally one would like each founder to have a very large and equal number of offspring. This not only maximises retention of gene diversity but would also preserves the existing allele frequencies. In other words, one would ideally like to "stop" selection such that the gene diversity that is available for reintroduction is the same as that collected from the wild generations earlier.

Real life is of course a different matter. Some founders will be more prolific than others. Allele frequencies will change (and some alleles may be lost) due to a combination of genetic drift (i.e., chance) and some individuals having better adapted to life in captivity. Whereas the emphasis is very much on maximising growth rate during the growth phase of the population, during the carrying capacity stage attention should be paid to correcting inequalities that have developed in the founder representation (Lacy, 1994).

In order to achieve this, the technique that is currently employed by the zoo and aquarium community is to base the pairings of the animals (i.e., who breeds with whom) on their mean kinship value. Mean kinship is a measure of the relatedness of an individual to every living individual in the population (Ballou, 1991; Ballou & Lacy, 1995). It is calculated as the average of the coefficients of kinship of an animal with every animal in the population. The coefficient of kinship between a pair of animals in turn is the inbreeding coefficient of any offspring produced by that pair of animals. Priority for breeding is given to individuals with low mean kinship values (and few relatives). After all, if such an individual should die before it gets a chance to breed, there are very few other individuals in the population that share some of its genetic variation and can help pass this on to the next generation. Chances are high that alleles only present in this individual will be lost from the population. For an individual with a high mean kinship value, chances are high that the majority of its gene diversity is also present in its many relatives. Individuals with high mean kinship values are therefore given lower breeding priority. Furthermore, efforts should be made to combine individuals with similar mean kinship values. If an

animal with low mean kinship would be combined with one with high mean kinship, the resulting offspring would be half important and half not important. Every time this offspring is bred, not only the rare alleles are spread, but also the already common alleles of the individual with high mean kinship value (Wilcken & Lees, 1998). The offspring is also going to be related to many individuals in the population which will make it harder to find breeding opportunities for it that do not result in inbreeding.

Apart from basing breeding priority and pair combinations on mean kinship, it is also important to minimise the level of inbreeding in a population. Inbreeding not only increases the level of homozygosity and reduces the amount of gene diversity retained, it often also causes inbreeding depression (a reduction in fitness of the inbred individual), partly due to an increased probability for homozygosity of recessive deleterious or lethal alleles (Frankham et al., 2002). Inbreeding depression may express itself in many forms, some of which may not be immediately obvious unless one consciously sets out to investigate them, e.g. reduced juvenile survival, reduced adult survival, less successful mate acquisition, lower social dominance ranking of inbred individuals, increased sensitivity to infections, reduced fertility, increased bilateral asymmetry, lower resistance to environmental stresses, etc. Despite earlier skepticism about the importance of inbreeding depression in wildlife populations, numerous wild populations have now been shown to suffer from inbreeding depression (Roelke et al., 1993; Crnokrak and Roff, 1999; Dietz et al., 2000; Sunquist and Sunquist, 2001; Frankham et al., 2002; Keller and Waller, 2002; Pimm et al., 2006). Captive populations too have been shown to suffer from inbreeding depression (Ralls and Ballou, 1986; Ralls et al., 1988; Boakes et al., 2007).

The level of inbreeding depression significantly influences the extinction risk of a population (O'Grady et al., 2006), and it would be dangerous and unwise to presume any population will be safe from inbreeding depression. In addition, populations with a high level of inbreeding that appear to be coping well enough in captivity may have significantly lower success rates upon reintroduction (i.e., in a more challenging environment) compared to non-inbred released individuals. For example, inbred white-footed mice *(Peromyscus leucopus)* showed significantly lower survival upon reintroduction than non-bred individuals (Jiménez et al., 1994).

Inbreeding is 'reversible', meaning that when an inbred individual is mated with an unrelated animal, the resulting offspring are no longer inbred. For this reason, it may sometimes be preferable to allow a modest level of inbreeding among low mean kinship animals, rather than pairing unrelated individuals with very different mean kinship levels. Naturally, these theoretically ideal breeding strategies for captive management need to be 'married' with the specific social and life history strategies of the species.

Choosing individuals to reintroduce

The modelling by Lacy and Vargas (2004) showed that the Iberian lynx *ex situ* population may be able to supply modest numbers of individuals for reintroduction after 8 years, and after 12 years, 12 to 13 cubs could be supplied for reintroduction per year, while still maintaining the required (for maintenance of gene diversity) nucleus population of 60 breeders (Figure 1). Especially for species as critically endangered as the Iberian lynx, the temptation is large to start reintroducing as soon as possible. Reintroductions are risky and the probability for whole or partial failure of particularly the first release attempts is high. The urge to reintroduce is therefore best contained until the required, secure, reliable nucleus breeding population has been obtained (Lacy, 1994). Rather than limiting breeding to keep the population at carrying capacity, growth can at this stage be maintained and the "surplus" animals used for reintroduction.

This automatically leads to the question, which individuals are best chosen for reintroduction? What are the best genetic criteria upon which to base this choice?

It is obvious that reintroduced individuals have to help improve the genetic and demographic health of the wild population. From this point of view, the reintroduced individuals need to be physically healthy, have a high reproductive fitness, a high gene diversity, and as low an inbreeding level as possible. What is often forgotten however is that the removal of the animals destined for reintroduction should also not compromise the genetic and demographic health of the nucleus *ex situ* breeding population. For that reason, individuals for reintroduction are preferably those that benefit the gene diversity of the wild population (i.e., have few relatives in the reintroduced population), but are genetically overrepresented in the captive population (Frankham et al., 2002). As reintroductions are risky however, first attempts are best tested with animals that are overrepresented

in both the wild and captive populations. Once release methods have been tested and fine tuned and once survival and reproduction in the reintroduced population have improved, animals more valuable to the wild population can be added (Frankham et al., 2002).

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References

Ballou, J.D., 1984. Strategies for maintaining genetic diversity in captive populations through reproductive technology. Zoo Biology 3, 311-323.

Ballou, J.D., 1991. Management of generic variation in captive populations, in: Dudley, E.C. (Ed.), The unity of evolutionary biology, proceedings IV International Congress on Systematics and Evolutionary Biology. Diodcorides Press, Portland, Oregon, pp. 602-610.

Ballou, J.D., Lacy, R.C., 1995. Identifying genetically important individuals for management of genetic diversity in pedigreed populations, in: Ballou, J.D., Gilpin, M., Foose, T.J. (Eds.), Population Management for Survival and Recovery. Columbia University Press, New York, pp. 76-111.

Boakes, E.H., Wang, J., Amos, W., 2007. An investigation of inbreeding depression and purging in captive pedigreed populations. Heredity 98, 172–182.

Crnokrak, P., Roff, D.A., 1999. Inbreeding depression in the wild. Heredity 83, 260-270.

Crow, J.F., Kimura, M., 1970. An introduction to population genetics theory. Harper and Row, New York.

de Boer, L.E.M., 1989. Genetics and breeding programmes – genetic guidelines and their background for EEP coordinators. National Foundation for Research in Zoological Gardens, Amsterdam.

de Boer, L.E.M., 1993. Founder representation: how to deal with it? National Foundation for Research in Zoological Gardens, Amsterdam.

Delibes, M., Rodríguez, A., Ferreras, P., 2000. Action plan for the conservation of the Iberian lynx *(Lynx Pardinus)* in Europe. Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention). Nature and environment, No. 111. Council for Europe Publishing.

Dietz, J.M., Baker, A.J., Ballou, J.D., 2000. Demographic evidence of inbreeding depression in wild golden lion tamarins, in: Young, A.G., Clarke, G.M. (Eds.), Genetics, demography and viability of fragmented populations, Cambridge University Press, Cambridge, pp. 203-211.

Frankham, R., Ballou, J.D., Briscoe, D.A., 2002. Introduction to conservation genetics. Cambridge University Press, Cambridge.

Jiménez, J.A., Hughes, K.A., Alaks, G., Graham, L., Lacy, R.C., 1994. An experimental study of inbreeding depression in a natural habitat. Science 266, 271-273.

Keller, L.F., Waller, D.M. 2002. Inbreeding effects in wild populations. Trends in Ecology and Evolution 17, 230-241.

IUCN, 1995. IUCN/SSC Guidelines for re-introductions. IUCN, Gland, Switzerland.

Lacy, R.C., 1994. Managing genetic diversity in captive populations of animals, in: Bowles, M.L., Whelan, C.J. (Eds.), Restoration of Endangered Species. Cambridge University Press, Cambridge, pp. 63-89.

Lacy, R.C., Vargas, A., 2004. Informe sobre la gestión Genética y Demográfica del Programa de Cría para la Conservación del Lince Ibérico: Escenarios, Conclusiones y Recomendaciones. Conservation Breeding Specialist Group, Apple Valley (MN) and Ministerio de Medio Ambiente, Madrid.
http://www.lynxexsitu.es/genetica/documentos_genetica.htm.

Mace, G.M., 1986. Genetic management of small populations. International Zoo Yearbook 24/25, 167-174.

Nunney, L., Campbell, K.A., 1993. Assessing minimum viable population size: demography meets population genetics. Trends in Ecology and Evolution 8, 234-239.

O'Grady, J.J., Brook, B.W., Reed, D.H., Ballou, J.D., Tonkyn, D.W., Frankham, R., 2006 (in press). Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. Biological Conservation (in press).

Palomares, F., Revilla, E., Gaona, P., Fernández, N.,Giordano, C., Delibes, M.. 2002. Efecto de la Extracción de Linces Ibéricos en las Poblaciones Donantes de Doñana y Sierra de Andújar para Posibles Campañas de Reintroducción. Informe sin publicar preparado para la Consejería de Medio Ambiente de la Junta de Andalucía. Noviembre, 2002.

Pimm, S.L., Dollar, L., Bass Jr, O.L., 2006. The genetic rescue of the Florida panther. Animal Conservation 9, 115-122.

Pollak, J.P., Lacy, R.C., Ballou, J.D., 2007. Population Management 2000, version 1.213. Chicago Zoological Society, Brookfield, IL.

Ralls, K., Ballou, J., 1986. Captive breeding programs for populations with a small number of founders. Trends in Ecology and Evolution 1, 19-22.

Ralls, K., Ballou, J.D., Templeton, A.R., 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. Conservation Biology 2, 185-193.

Reed, D.H., Frankham, R., 2003. Correlation between fitness and genetic diversity. Conservation Biology 17, 230-237.

Rodríguez, A., Delibes, M., 1992. Current range and status of the Iberian lynx *Felis pardina* Temminck, 1824 in Spain. Biological Conservation 61, 189-196.

Roelke, M.E., Martenson, J., O'Brien, S.J., 1993. The consequences of demographic reduction and genetic depletion in the endangered Florida panther. Current Biology. 3, 340-350.

Sunquist, M.E., Sunquist, F., 2001. Changing landscapes: consequences for carnivores, in: Gittleman, J.L., Funk, S.M., Macdonald, D., Wayne, R.K. (Eds.), Carnivore conservation. Cambridge University Press, Cambridge, pp. 399-418.

Thomas, C.D., 1990. What do real populations tell us about minimum viable population sizes? Conservation Biology 4, 324-327.

Thompson, E.A., 1994. Genetic importance and genomic descent, in: Ballou, J.D., Foose, T.J., Gilpin, M. (Eds.), Population Management for the Survival and Recovery. Columbia University Press, New York.

Vargas, A., Martínez, F., Bergara, J., Klink, L.D., Rodríguez, J., Rodríguez, D., 2005. *Ex situ* Iberian lynx project update. International Zoo News 62, 480-481.

Wilcken, J., Lees, C. (Eds.), 1998. Managing zoo populations: compiling and analysing studbook data. Australasian Regional Association of Zoological Parks and Aquaria: Sydney, Australia.

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