Cloning advances and challenges for conservation

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Although controversy surrounds cloning efforts, the cloning of animals to assist efforts to preserve genetic variation in support of endangered species conservation efforts has attracted serious interest. A recent report by Loi *et al.* describing the cloning of a mouflon (a species of wild sheep) in a domestic sheep surrogate points to potential conservation opportunities and additional challenges in the evaluation of appropriate technologies for present and future efforts to conserve gene pools of endangered species.

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Each major report about cloning involving somatic nuclei brings new insights and new debates. As the controversy around human cloning expands [1,2] there is a diversity of opinion regarding the potential of cloning for the conservation of endangered species [3–5]. It is important to evaluate separately the issues surrounding human cloning and those of animal cloning.

With respect to predictions for loss of species, technologies for assisted reproduction, such as artificial insemination, embryo transfer and cloning from somatic cells, have been advocated as technologies that could contribute to conservation of biological diversity [6].

Successful cloning of an endangered sheep

Discussion of the application of cloning technology to conservation efforts for endangered species [3] was an immediate result from Ian Wilmut's 1997 report of the cloning of Dolly [7]. Although reports of embryo development [8,9] and newborn animals have appeared as a result of cloning technology, the recent report of surprising success in cloning mouflon (a species of wild sheep) [10] is notable for several reasons. The success rate was much greater than when the domestic sheep, Dolly, was cloned. A higher proportion of embryos (constructed by nuclear transfer to enucleated domestic sheep ova) developed *in vitro* to blastocysts and, subsequently, to pregnancies and live birth in surrogate dams than previously reported [7,11]. It is also noteworthy that the donor nuclei were obtained from dead donor mouflon. These rather unexpected findings might serve as the basis for additional studies to help identify factors contributing to the rate of success. Studies of telomere length were not reported and future work in this area will be of interest.

From a theoretical perspective, there is reason to believe that cloning can assist in the preservation of genetic diversity in precariously small populations. The cloned animals, as individuals, might serve as conduits for the retention of genetic variation otherwise lost. There are many vulnerable and endangered forms of sheep (including forms of argali, urial, desert bighorn and Marco Polo and snow sheep) for which this technology could be considered in defined programs of gene pool preservation.

Objections to the use of cloning technology for conservation focus on inefficiencies of the current process, impracticalities involved in applying these techniques to non-domestic ova donors and surrogate dams, and the lack of fitness for survival of cloned animals in the natural environment. Fitness concerns are heightened owing to the use of domestic surrogates that fail to impart appropriate behavioral attributes for cloned offspring that will interact with others of their species raised by conspecific mothers (i.e. mothers of the same species).

Defects in cloned animals

Abnormal morphology and lack of developmental success in cloned mice is associated with abnormal regulation of imprinted genes [12]. Thus, imprinted genes in successfully (and unsuccessfully) cloned animals have been investigated in detail [13]. As the specific loci subject to imprinting have been modified in the course of mammalian evolution [14,15], further studies of the evolution of imprinting and imprinted genes in mammals might also provide useful insights. Active management of deprogramming differentiated nuclei and epigenetic effects is an area of active investigation that might eventually increase the fitness of cloned animals.

As discussions of the fitness of clones continue, the question is not so much whether Dolly has arthritis as whether her descendants have a reduction in their fitness associated with the deprogramming of her genome that facilitated her development from the nucleus of a differentiated cell. Also, apparently normal cattle have resulted from cloning [16]. The over-arching concern for the genetic continuity of a species rather than for the fitness of a single individual is a crucial difference between application of cloning technologies to endangered animals in comparison to humans.

Cloning as a tool for assisting in conservation of gene pools

When considering the potential role of cloning to help the conservation of endangered species, a crucial point is whether cloning represents a functional technology suitable to the management of gene pools. The changes in gene pools of vulnerable populations becoming endangered will limit viability of some populations with grave prospects for recovery. The potential to modulate loss of genetic variation in small populations undergoing sexual reproduction by incorporating genetic variation from unrelated individuals or individuals of known genotype or phenotype from preserved cell nuclei offers a form of intervention previously unimaginable in the animal breeding or conservation breeding context. Although we would prefer to envision that, if intervention is required, some limited form of management will be sufficient to ensure the viability of populations in protected areas and, indeed, all suitable habitats, we can by no means say that this is assured.

Allelic diversity is lost owing to drift and, in small populations, the persistence of rare alleles becomes vulnerable to chance events. Practical intervention will probably consist of managing retention of genetic variation, including heritable attributes that are most likely yet to be identified. Deleterious loci might need to be detected and their frequencies managed in the population. Haplotype diversity might also be desirable to manage, and evidence for selection for some haplotypes might become apparent as a result of population studies.

Alteration in allele frequencies that could accumulate over generations as a result of differential selection and drift in a captive environment might be mitigated if founder

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and early generation individuals could be used for breeding to provide individuals for reintroduction and augmentation programs.

Cell banking and research should top the current agenda

We are probably not at the stage where cloning technology is ready to be applied to maintain population viability or conserve species for which the technology is available and, in any case, cloning is no panacea. However, in the struggle to maintain self-sustaining populations cloning might have a future role more significant than present technology suggests. Looking to the future, there will probably be instances in which cloning technology can make a crucial difference for some species. Although it might be decades from now that answers become clear, it is apparent that access to declining levels of genetic diversity is more readily available now than in the future. Additional research should be welcomed and evaluated in the context of conservation.

Anticipation of potential benefits to be derived from the strategic use of cloning technology will require a broad understanding of its limitations in the context of specific conservation goals. For which species might cloning technology be considered? Where might the most significant benefit be derived from initial efforts? Surely, cells that might be later used for a variety of purposes, including cloning, should be collected as opportunity allows – for many species in peril this needs to be done sooner rather than later. We will not be able to explore the potential of cloning without additional studies, which could be focused on development of a strategic tool for conservation management of small populations. Such studies will require access to cells that have been previously banked from species for which loss of genetic variation is considered to be detrimental to the maintenance of a self-sustaining population. There are few sources of such cells because, with a few exceptions, banking cells from small populations of endangered animals has not been undertaken. These exceptional collections offer much in the way of resources that might be used in evaluating the circumstances in which cloning technology might offer practical conservation benefits. Delaying such experimentation will forestall the collection of information crucial to the evaluation of cloning technology for targeted management of small populations for conservation.

Planning for the future

Certainly, a concerted effort involving collaborations of field biologists familiar with the status of threatened and endangered species with reproductive scientists, geneticists, and others with expertise and resource banking should be undertaken to match conservation and technological opportunities. Identification of the taxa at risk and the systematic collection of samples as opportunities arise, consistent with the conservation management of threatened and endangered species, offer increased opportunities for preventing extinction and for the preservation of gene pools.

In the future, even if efforts to establish banks of cells from endangered species are viewed as a needlessly pessimistic strategy, these cell banks will be of great use for a variety of biological studies that will increase the understanding of the natural world and its evolution. It has been suggested that an abrogating effect of the effort to bank cells is the establishment of unrealistic and unattainable programs for effective conservation and insufficient diligence to ensure preservation of sufficient natural habitat for conservation of biodiversity. However, an effort in genetic resource banking for endangered species serves the interests of future generations irrespective of the application of cloning technology. Furthermore, cell-banking efforts are not envisaged as efforts *in lieu* of *in situ* conservation but as supporting efficiencies and informed decision making capabilities that assist *in situ* conservation efforts.

Summary

The successful cloning of a mouflon from cells of an animal found dead in the field again raises the possibility that cloning technology can assist with the management of endangered species. Although the fitness of cloned animals remains a subject of controversy, the potential of cloned individuals to contribute to the retention of genetic variation in small populations provides an opportunity for this technology to contribute to conservation efforts. Increased research, targeted application of the technology and an expanded effort to bank cells are indispensable before this technology will make a significant impact on small population management for conservation.

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