

COMMENTARY

The Potential Use of “Cloning” in the Conservation Effort

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The recent demonstration of the feasibility of cloning adult mammals has opened a variety of new research ideas for conservation-minded biologists. It requires much work ahead but the potential rewards are considerable. We stand to learn much of the reproductive biology of many species and, in the end, may find it easier for long-term conservation practices than is provided by other currently practiced methodologies. As discussed for earlier biotechnical advances [Lasley and Anderson, 1991], cloning cannot replace current practices of conservation of wild animals. It can be conceived, at best, as another desperate effort to salvage what is being lost. Indeed, it has the potential of minimizing loss of genetic resources as well as rescuing already lost genetic material; these may be its greatest assets.

Cloning may be defined as asexual reproduction, or as the creation of genetically identical individuals. Proliferation of identical fragments of DNA (molecular cloning) is also referred to as cloning, but is clearly different from somatic cloning of organisms. The idea of cloning is not new. It has been practiced by grafting of fruit trees for millennia and, in some mammalian species, it is the normal mode of reproduction. Thus, the nine-banded armadillo (*Dasypus novemcinctus*) regularly produces monozygotic quadruplets and its Argentinean cousin, the mulita, produces even more identical offspring by a similar segregation of blastomeres shortly after implantation [Newman et al., 1909]. In most other species, splitting of the early egg is a moderately frequent occurrence whose cause is not understood. Some investigators now consider that “cloning” represents the transfer of nuclei into oocytes [McKinnell, 1978], a technique delineated in detail for mice by McGrath and Solter [1983]. Experimentally, one form of the cloning of embryos has been achieved for instance by Spemann [1901–1903] in *Triton* eggs through splitting of early embryos into identical multiples. More recently, in a variety of domestic animals monozygotic multiple

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offspring have been produced by segregating blastomeres of morulae. This is feasible at least to the 32 cell stage [review, Seidel, 1983]. Still more recently, new techniques of "cloning" have come into discussion following the experiments by Gurdon [1986] who transplanted nuclei from tadpoles into frogs and vice versa—with poor results (the tadpoles usually died).

Now, this topic has received new attention following the report by Wilmut et al. [1997] who reported the production of a sheep, "Dolly", by the transplantation of a mammary cell nucleus from an adult ewe to the enucleated oocyte of another sheep. This has suddenly become the popular type of "cloning" and is understood as such by the general public. The enucleate ooplasm with its mitochondria and other organelles in this achievement served as sufficient medium in which to express the genetic heritage of a nucleus derived from a growth-arrested, differentiated somatic cell. This process could also be viewed as the production of a nucleus/cytoplasm chimera. The immediate reaction to this experiment was a ban on human cloning [Lancet, 1997] because it raised the specter of producing "clones" of undesirable individuals. But also, suggestions for the consideration of potential medical benefits derived from potential human cloning have been forthcoming; they suggest the possibility of cures for maternal mitochondrial diseases [Orgel, 1997]. It should be noted that cloning in this newest sense of its meaning was not deemed to be a procedure for the production of large numbers of genetically identical sheep but rather, the production of individual lambs to gain a better understanding of embryonic regulatory phenomena. In such applications, using techniques that could be applied, the introduction of a diploid nucleus into an enucleate egg, might thus be aimed to produce only one individual. We now ask: is there a place for cloning in the propagation of endangered animals in the conservation efforts of zoological gardens?

We should state at the outset that the subsequent considerations are not in the least to minimize or supplant current efforts of conservation practices. They obviously should continue, if not be expanded. Nevertheless, each new species that becomes a member of an *ex situ* conservation program has cost factors attached. The number of taxa to be included in our Species Survival Programs is limited by space and cost of maintenance of animals [Snyder et al., 1996]. The application of cloning technology has the potential of ameliorating both issues because of the possibility of long-term cryopreservation, as will be discussed. Potentially then, the number of taxa to be managed could be expanded.

One may argue that *ex situ* conservation inappropriately impacts on the primary goals of conserving species through maintenance of functioning ecosystems. To the extent that the global conservation effort has already recognized the supportive role of *ex situ* conservation through the Convention on Biodiversity for example [Ryder, 1995], expanding the technical capacity of this component poses risk only if the overall priority of conservation efforts should be changed. These priorities are the purview of governments which may be primarily concerned with human welfare. In the face of conservation actions that are not now wholly effectual, the use of new technologies stands among the greatest hopes for the rationalization of irreconcilable goals for conservation and development.

It is unlikely that cloning as discussed here could be applied to long-extinct taxa. We cannot bring back the quagga because no viable cells were saved. Furthermore, species whose reproductive physiology and endocrinology are poorly under-

stood are unlikely to be easily employed in conservation efforts of this sort. There is hope, however, that progress will be made.

While the demonstration by Wilmut et al. [1997] that an adult mammalian mammary cell can serve to transfer the genetic material to a new offspring is remarkable, it should be recalled that most of the 200+ transfers failed and that some abnormal embryos were also observed. No doubt, the technique will be improved and much will be learned in future experiments about the cellular phenomena that allowed this success. Thus, is the type of nucleus transferred essential or can any adult cell serve as donor? Must the donor cell be in the inactive phase of mitotic division? Is an electric "shock" needed for activation? What stage of recipient oocyte is required for successful propagation, and so forth? Additionally it is of concern to understand whether the presumed acquisition of mutations (possible telomere shortening for instance) of the donor cell nucleus limits the life span of the cloned animal. Perhaps the success with serial transplantation of skin [Krohn, 1962] and pancreas [Lee et al., 1997] provides encouragement in this regard, as the transplanted tissues showed little evidence of degenerative or aging changes, even though the normal rodent life span was significantly exceeded in the transplants. The major limitations in the serial pancreatic transplantation was a degeneration of the vascular supply; the pancreatic tissue showed no deterioration. Thus, perhaps at this juncture there is no need to fear a premature aging of the cloned animal.

Embryonic stem cells have recently been harvested from human neonatal umbilical cord blood for ultimate reimplantation [Silberstein, 1996]. Some success has been achieved in this technique and one may thus ask whether the preservation of placental tissue cells should also be advocated. This may have the advantage of being perhaps more uncommitted cellular tissue, with a potentially greater success for cloning. Moreover, not all embryos grow up and additional genetic material could thus be salvaged. On the other hand, current evidence suggests that placental cells are paternally imprinted, the embryo having maternal imprinting [Wilson et al., 1993]. Thus, would this as yet poorly understood imprinting be reversed when nuclear transplantation is done?

If all of these problems could be overcome, is it reasonable to expect that interspecific, let alone intergeneric exchange of nuclei could yield successful clones? A partial answer to this is provided by embryo transfers that have been performed to date [review, Kraemer, 1983]. In this respect, placentation may be a limiting factor [Hradecky et al., 1987]. Most species have significantly different placentas, structurally and often functionally. Who calls the shots of placentation - the nucleus or the cytoplasm? And how does imprinting work in this instance? The answers are as yet unknown but will be explored. At present, interspecific embryo transfer has been successful only when hybridization was feasible between the species in which embryo transfer is practiced. Needless to say, a better understanding of placentation among endangered - to be conserved - species is needed. But experimentation in this area is especially relevant when it comes to resurrecting species in which no immediate close relative may be available. Thus, how might it be feasible to clone a giant armadillo, surely a species that faces dire risk of extinction? It has never reproduced in captivity and cryopreservation of adult giant armadillo cells for future cloning is clearly still feasible. But which species can serve as a maternal "container" in which to grow such a cloned animal? Similar examples are easily enumerated. Thus, would a cloned Sumatran rhinoceros grow in the uterus of a recipient white rhinoceros,

even if the reproductive parameters of both species were fully understood? The answers are not yet known, but it is imperative that answers be sought to such questions. In part, this may also provide an additional justification for the conservation of cells in optimal condition, as advocated by Russell [1978] and practiced in the "Frozen Zoo" of CRES at San Diego Zoo and elsewhere. Indeed, somewhat similar thoughts were expressed by Wildt [1992] in his remarks on "Genetic Resource Banking", even though the cloning technique had not then been perfected. This contribution not only addresses the need for global action but, importantly, it seeks to engage the advanced technology of domestic animal reproduction specialists. The progress in this field surpasses the capability of most zoos; thus, assistance from researchers and practitioners will be needed in this endeavor.

If the technique of cloning adult cells as done in "Dolly" were to become a practical resource for conservation purposes, could one not then envisage that far fewer animals might need to be bred and kept in large herds, just to conserve their genetic variability? In this regard, application of cloning technology stands to have a long-term benefit of increasing retention of genetic variation in small populations. Consideration of preservation of gene pools is a potentially major aspect of mammalian cloning, as now understood. The future for clonable species would clearly be better than that for animals that cannot be cloned. There is clearly already now a limitation of space for the maintenance of large herds of animals just in order to maintain the necessary gene pool for extended periods [Soulé et al., 1986; Conway, 1986]. Under this limitation the inherent conflict between accommodating increasing numbers of candidate taxa with the benefits of Species Survival Plans (SSPs) and meeting the criteria of these plans for conserving genetic variability may be substantially ameliorated, even at future times, if material for genetic propagation (such as somatic cells) is collected now. Primary considerations in establishing the steady-state census number for SSP efforts include the number of founders and the magnitude of genetic drift in these small populations. Both these considerations can be restructured with the availability of successful cloning technologies. If founder individuals are available for cloning, then the possibility exists to have zero net loss of genetic variation, provided that sufficient appropriate surrogate dams are available. Managers of small populations would include a combination of sexual reproduction and cloning to manage retention of genetic diversity and recombination of genotypes in their populations. When access to founders is not available, access to the genome of early generation descendants of founders might be incorporated into breeding programs even if knowledge of husbandry and animal health is insufficient to secure their desired reproductive contributions to a breeding program during their lifetimes. Many appropriate scenarios will immediately come to mind when conservationists consider the applicability of this new modality. Moreover, if cloning were to become utilized, it would focus attention on the surrogate dams, including their behavior. The importance of mother/infant relationships, for instance, especially with regard to reinforcing appropriate natural behaviors adaptive to the wild for reintroduction purposes would receive increased scrutiny. The positive side of this is that females that impart desirable behavioral traits to their offspring could do so to genetically unrelated individuals. The considerable effort to understand animal behavior in zoological parks should benefit from cloning technology as an experimental tool to test hypotheses regarding effects of genotype versus environment or sociobiological theory.

Appropriate first steps in the development of, and application of, cloning in the propagation of endangered species and conservation of their gene pools are logical extensions of the efforts of Wilmut and his colleagues [1997]. These might involve using banked somatic cells, e.g., fibroblasts as a source of nuclei for injection into enucleate ova from the same species or from species previously known to serve as surrogate dams for the species serving as nuclear donor. Thus gaur [Stover et al., 1979] and banteng [Wiesner, H., personal communication] calves have been born to domestic cattle. Their nuclei might be tested in cow ova. Banked somatic cells from small exotic felids might serve as nuclear donors utilizing ova from domestic cats, and tigers of untraceable pedigree or those otherwise surplus to SSP propagation efforts could serve as surrogate dams and/or donors of ova to be enucleated and injected with prepared somatic nuclei of critically endangered tiger subspecies, such as the South China tiger. In short, serious consideration should be given to exploit and advance this exciting technology, rather than dismissing it out of hand.

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