

# GENETIC AND PHENOTYPIC SIMILARITY AMONG MEMBERS OF MAMMALIAN CLONAL SETS

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“Your dog [pronounced *dawg* by most Texans] is dead.” This is the succinct answer given by Mark Westhusin, in a presentation at the 2001 annual meeting of the International Embryo Transfer Society, to the question of “How similar would a clone be to my pet dog who just died?” “You’re history” might be a similarly appropriate response to people who wish to clone *themselves* to achieve immortality: there are myriad scientifically defensible applications of cloning, but recreating an animal, except in a genetic sense, is not one of them. Perfect genetic identity is actually unachievable, although one can get close for some practical purposes. An example is cloning a valuable bull; most valuable bulls are kept for their sperm production, and a clone will produce a population of sperm identical to that of the original animal, except for mutations and the mitochondrial genome. Phenotype is of little consequence as long as fertile sperm are produced.

Phenotypic identity is most elusive. Phenotype obviously is determined by genotype, environment, epigenetic effects (often with a chance component), chance itself, and interactions of all these factors (Seidel, 1983). It has become increasingly clear over the past few decades that chance epigenetic effects can have substantial influence for some traits (Reik *et al.*, 1993; Finch and Kirwood, 2000). These ideas are the basis for this chapter.

## DEFINITION OF CLONING

The word “clone” is derived from the Greek word for twig. This etymology is easily understood, because our species has known for millennia that many species of plants can be propagated from somatic tissues such as twigs. Clonal reproduction is routine for dozens of domesticated plants, such as potatoes, and is common in nature as well, e.g., groves of aspen trees. A variation that is fairly common in nearly all mammalian species is identical multiplets; the incidence of identical twins and triplets, etc., is low in most species, but in others, such as armadillos, this occurs with every pregnancy. For practical purposes, identical multiplets are the “gold standard” of the maximum degree achievable of phenotypic identity of mammals. None of the methods of cloning will achieve greater identity, and cloning by nuclear transplantation/cell fusion results in considerably less identity.

But are identical twins or triplets clones? Perhaps terms such as “clone” or “cloning” should be used less frequently, and instead more specific descriptions should be used, embracing concepts such as making genetically identical sets by blastomere separation, splitting postcompaction embryos, or nuclear transplanta-

tion. Some scientists define nuclear transplantation/cell fusion as "true cloning," suggesting that other procedures should not come under the umbrella term "cloning," even though some of these other procedures result in animal sets that are more identical, compared to sets produced by nuclear transplantation/cell fusion.

## CYTOPLASMIC GENETICS

The clearest example of cytoplasmic inheritance in animal cells is the mitochondrial genome. The approximately 16,000-base-pair circular mitochondrial genome has genes for ribosomal RNAs, transfer RNAs, and approximately a dozen mitochondrial proteins (Cummins, 1998). Although the vast majority of mitochondrial proteins are specified by chromosomal genes, the mitochondrial genome has considerable variability, and mutates more readily than chromosomal genes, in part due to poor proofreading during DNA synthesis (Cummins, 1998). The resulting mutations are the source of considerable (cytoplasmic) genetic disease, as well as phenotypic variation in normal mitochondrial function. Although there are rare exceptions (Cummins, 1998), for the most part, mitochondrial genomes are inherited via the maternal ooplasm. The relatively few mitochondria introduced by the sperm usually degenerate, and in any case become so dilute that they usually would not end up in the relatively few cells of the blastocyst that differentiate into the resulting animal.

The maternal lineages of mitochondria in the recipient oocyte and in the donor nucleus often will be different. Simply by the process of dilution, the animal resulting from cloning procedures using cell fusion usually ends up with mitochondrial genes of the oocyte, not the donor nucleus. Of course, mitochondria will be heteroplasmic initially; sometimes this situation persists, and rarely the donor mitochondria out-compete the recipient oocyte mitochondria, but usually the mitochondria of the recipient oocyte prevail (Evans *et al.*, 1999). It is also possible that donor mitochondria do not survive well in ooplasm because of the very specially differentiated state of mitochondria in oocytes (Cummins, 1998). Experiments to sort this out are progressing, and it is obvious that answers will have a statistical quality rather than a simple outcome. Note that in the future, it is likely that heteroplasmy of mitochondrial genomes can be eliminated by selective elimination of donor or recipient mitochondria by chemical or other means.

Cytoplasmic inheritance also occurs for centrosomes, usually via the sperm in mammals (Stearns, 2001). To my knowledge, no cytoplasmic nucleic acid sequence information is involved, but the semiconservative nature of centriole duplication (Stearns, 2001) does have implications for cloning, particularly if procedures become more sophisticated. For example, centrosomal material might be provided from sperm parts rather than from donor cell cytoplasm.

Cytoplasmic inheritance of viruses occurs in some situations. In these cases, there is a nucleic acid sequence specifying a cytoplasmic component.

## EPIGENETIC EFFECTS

A simple definition of "epigenetic" is elusive, although many epigenetic qualities are captured by the definition: different genetic and/or phenotypic outcomes from the same DNA sequence. During embryonic and neonatal development, there clearly are dozens and perhaps hundreds of epigenetic events that influence phenotype differently in genetically identical animals. Several examples of epigenetic phenomena are given in Table 1. There is also evidence of epigenetic instability when embryonic stem cells are used as donor nuclei (Humpherys *et al.*, 2001), and possibly when there are several rounds of nuclear recycling (Peura *et al.*, 2001).

One of the most obvious differences between genetically identical animals is coat color patterns in strains that are not one solid color. Although the broad pattern

**Table 1 Examples of Epigenetic Effects**


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Migration patterns of melanoblasts and primordial germ cells during fetal development
Random inactivation of X chromosomes in female mammals
Numbers of beta cells in the endocrine pancreas, or oocytes in ovaries
Gametic imprinting
Telomere length
Differential methylation of cytosines in DNA of different tissues

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of coloration is similar among genetic identicals, the specific pattern can be quite different. This is mostly a consequence of how melanoblasts, originating from the neural crest, invade hair follicles; there obviously is not a genetic instruction for each follicle (Seidel, 1985). Similarly, the numbers of primordial germ cells that invade each gonad have a statistical quality (Tam and Snow, 1981), as also would be expected for many other aspects of embryonic development. There are many such examples, some of which easily distinguish one identical twin from another. One example is iris pigmentation patterns (Daugman, 2001). An extreme example is monozygous human twins with different hair color, thought to be due to differential melanoblast migration (Gringras, 1999), illustrating the imprecision of the term "identical twins."

During organogenesis in female mammals, the maternal or paternal X chromosome is inactivated in individual cells (Gardner *et al.*, 1985); once this decision is made, progeny of cells maintain the initial inactivation, either the maternal or paternal X chromosome (Eggan *et al.*, 2000). By chance, a given tissue or organ from genetically identical females may have more cells with paternal or maternal X chromosomes inactivated. This is perhaps best illustrated with tricolor female cats, in which red or black hair color is an elegant marker for which parental X chromosome is active in melanocytes of any given area of cat skin. Identical twin tricolor cats would have very different patterns of red and black due to superimposition of two epigenetic effects: X-chromosome inactivation patterns and melanoblast migration. Similar, less easily visualized epigenetic phenomena occur frequently during embryonic development (Reik *et al.*, 1993; Finch and Kirwood, 2000).

Just as there are genetic  $\times$  environmental interactions, there are epigenetic  $\times$  environmental interactions, nicely illustrated by the integrating work of Barker (e.g., Barker, 2000). The main concept is that maternal nutrition during gestation can have profound effects on phenotype for chronic diseases decades later. Maternal undernutrition, for example, leads to increased incidence of diabetes, heart disease, and depression. This has an epigenetic basis via the numbers of cells that are allocated to different tissues, or how they differentiate within tissues. Doubtless these events are mediated via modifications of histones (Berger, 2001), such as methylation and acetylation, or differential methylation of cytosines in the DNA of these tissues. Although maternal undernutrition or overnutrition has consequences, one would not expect identical sequellae in each member of a set of clones.

Gametic imprinting, by which expression of genes depends on sex of parental origin rather than DNA sequence, is one of the more fascinating examples of epigenetic inheritance (Surani *et al.*, 1986; Moore, 2001). Theoretically, this should not lead to epigenetic or phenotypic differences among clones. However, there are some indications that imprinted genes become reprogrammed inappropriately during nuclear transplantation to clone mammals (Moore, 2001; Wrenzycki *et al.*, 2001), possibly explaining some of the huge differences in the sizes of placentas and fetuses among genetically identical offspring.

Telomere length is another epigenetic trait that might be quite relevant in donor chromosomes. There is little debate that telomere length decreases with each mitotic cell division in many somatic tissues in a number of species, and thus decreases with aging. However, there is conflicting information on the degree of restoration of telomere length with cloning procedures. Results range from slight shortening (Shiels *et al.*, 1999) to no effect (Kubota *et al.*, 2000) to significant lengthening (Lanza *et al.*, 2000). These differences in findings could be due to cloning procedures, sources of donor nuclei, species, or chance with individual clonal sets. Some donors have been quite aged (Kubota *et al.*, 2000), but there have been no observations of premature aging reported to date. Perhaps none will occur; knockout mice for telomerase had to go through six generations to observe a phenotype (Blasco *et al.*, 1997) and mice have been recloned through six generations with no buildup problems (Wakayama *et al.*, 2000). The situation could be quite different, however, with longer lived species with shorter telomeres than are found in mice.

### UTERINE EFFECTS

All procedures for cloning mammals require embryo transfer, and thus exposure to the vagaries of different uterine environments. In species in which inbred lines are available, sets of recipients can be made essentially genetically identical. F<sub>1</sub> crosses often are used. They have the special advantages of hybrid vigor and being genetically identical sets without being inbred. However, these theoretical advantages are moot for most nonresearch applications because inbred lines are available for only a few species. The relatively expensive option now is available, however, of cloning animals to be recipients so that uteri of recipient sets are genetically identical. However, even these would likely be of different ages.

Litter-bearing species present numerous challenges for achieving identical environments within the same uterus, or between uteri. Litter size affects birth weight and gestation length, and thus stage of maturity at birth. Because of the unpredictability of pregnancy rates per embryo, cloning procedures exacerbate variability in litter size. When transferring embryos with low probabilities of survival, carrier embryos of a different genotype, but high probability of survival, are often used (sometimes provided by just mating the recipient). Although carrier embryos make litter size more uniform, they compete for implantation sites and affect the local uterine environment in various ways—for example, in placenta size.

When litters are large, there often are one or two runts of the litter. These frequently are from implantations crowded into the tips of uterine horns, where vasculature did not develop as well as in the rest of the uterus. The runt phenotype generally remains with the animal for life.

Normally, there is a spectrum of masculinity and femininity in litter-bearing animals that is due in part to the gender of fetuses implanted on either side of another fetus (vom Saal, 1989). An oversimplified view, which illustrates the point, is that an embryo in the miduterine horn could have two females, two males, or one of each sex for immediate neighbors. Apparently the hormones and other factors that neighbors secrete make a permanent difference in degree of gender phenotype. Nongender phenotypic effects, such as growth characteristics, likely also occur, but are largely unexplored.

Obviously, these kinds of effects are minimized with clonal sets of embryos in the absence of carrier embryos. However, they do become an issue if one is trying to copy a particular animal. One could imagine a female pig that was gestated next to two males, and therefore grew faster and was somewhat more aggressive in feeding. Litters of clones of this pig would not have those different gender-related neighbor-induced characteristics. These kinds of issues are especially relevant when attempting to copy extreme phenotypes, because they are not entirely genetically based.

Uterine variation also is present in monotocous species, and is exacerbated by twin gestations, whether fraternal or identical. Note that because of low pregnancy rates per embryo, twinning with embryo transfer is a frequent practice in cattle, and twin-clone gestations are not so unusual. Such gestations are shorter than normal and produce smaller calves than do singleton gestations. Birth weight also is affected by breed, parity, age, and size of recipients. Vertical transmission of pathogens can also affect offspring.

## NEONATAL ENVIRONMENT

A plethora of differences in neonatal environments can influence phenotype, ranging from nutrition to climate, from prevalence of disease-causing organisms to competition from siblings. All mammals require milk to thrive neonatally, and the amount and composition of suckled milk will vary greatly among recipients. Passive transfer of immunity via immunoglobulins in colostrum and/or across the placenta varies enormously. Maternal behavior also affects neonatal health, well-being, and socialization.

## LARGE-OFFSPRING SYNDROME

Large-offspring syndrome is one of the most spectacular examples of phenotypic variation within genetically identical clutches of cloned animals. This is illustrated for birth weight of calves cloned (Table 2) using blastomeres of morulae as donor cells (Garry *et al.*, 1996). Ironically, there often is more variation in birth weights within genetically identical clonal sets than among full sibs (Green *et al.*, 1996; Gärtner *et al.*, 1998). The syndrome was first described in the context of cloning by nuclear transfer by Willadsen *et al.* (1991) and was documented thoroughly by Wilson *et al.* (1995). It is poorly understood and frequently misunderstood. Large-offspring syndrome has been documented primarily in sheep and cattle (Sinclair *et*

**Table 2** Characteristics of Clonal Sets of Calves<sup>a</sup>

Set	Birth wt. (kg)	Gestation length (days)	Set	Birth wt. (kg)	Gestation length (days)
Set 1	30.9	271	Set 5	42.3 <sup>b</sup>	273
	30.9	279		47.3 <sup>b</sup>	288
	33.6	283			
	42.7	289			
Set 2	41.8	275	Set 6	37.3	291
	43.6 <sup>b</sup>	293		40.0	296
	55.5 <sup>b</sup>	289		45.5	297
	47.3 <sup>b</sup>	288		44.1	299
			46.8	310	
Set 3	44.5	283	Set 7	42.7	294
	53.6	293		40.0	295
	65.5	293			
Set 4	57.7	291	Set 8	32.7	273
	62.7	298		41.8	273
	54.1 <sup>b</sup>	293		30.0 <sup>b</sup>	282
				45.5	283
				46.4	289

<sup>a</sup>From Garry *et al.* (1996).

<sup>b</sup>Indicates calf died neonatally.

*al.*, 2000) and may not occur in all species, although it clearly can occur in mice (Eggan *et al.*, 2001). There may be multiple etiologies, but the primary lesion in most cases likely is an epigenetic disturbance of placental function (Hill *et al.*, 2000b; De Sousa *et al.*, 2001). Thus, fetuses may become abnormal solely due to the placental environment, much as occurs with macrosomic babies born to diabetic mothers.

What is terribly misleading about the syndrome is that the high incidence of neonatal death and morbidity of animals derived via cloning by nuclear transplantation often is ascribed to birthing difficulty due to large size. Far more serious are epigenetic disturbances of metabolism (e.g., Garry *et al.*, 1996), again most likely of placental origin and not correlated with size of offspring. The metabolic disturbances are exacerbated by an abnormal parturition process that is prolonged, among other problems. Also, the metabolic problems result in the ultimate in phenotypic variation, life or death, whether it be embryonic, fetal, neonatal, or postnatal death. In addition, the incidence of congenital abnormalities is increased (Hill *et al.*, 1999). That birthing difficulty due to size is not the fundamental problem is clearly illustrated by Garry *et al.* (1996), who derived cloned calves by elective cesarean section when parturition was imminent. Note that this is a common practice for cloned calves (e.g., Wells *et al.*, 1999) as well as for calves produced commercially by *in vitro* oocyte maturation, fertilization, and culture of embryos. Oddly, elective cesarean section a few days preterm actually decreases phenotypic variation among cloned calves.

Large-offspring syndrome and related problems occur in many situations (Khosla *et al.*, 2001) unrelated to cloning, albeit usually at a lower incidence. However, problems can be huge, just with *in vitro* maturation, fertilization, and culture of embryos. For example, in the study of Behboodi *et al.* (1995), seven of eight calves died neonatally, and Walker *et al.* (1992) had 20% losses of lambs compared to 3% for controls, just from culturing *in vivo*-produced embryos. *In vivo*-vs. *in vitro*-matured and -fertilized oocytes also can contribute to large-offspring syndrome (Behboodi *et al.*, 2001). With cloning, *in vitro* oocyte maturation and *in vitro* culture and embryo transfer are superimposed on the cloning procedures. Under some circumstances, effects may be multiplicative.

It is important to note that these epigenetic effects will decrease as more is learned. Removing serum from culture media can help (e.g., Thompson *et al.*, 1995) as can avoiding coculture systems. The problem will never disappear, because large-offspring syndrome occurs at a low incidence (<1% in most breeds) with naturally mating cattle, and can be exacerbated in numerous ways, such as hormone treatments and asynchronous embryo transfer.

## MUTATIONS

Because billions of base pairs per cell are replicated to produce hundreds of billions to trillions of cells in an animal, there will be some mistakes. Other mutations are environmentally induced—for example, from gamma rays or from intracellular peroxidation events. The majority of consequential mutations are corrected or eliminated by cell death. Nevertheless, mutations are bound to accumulate, particularly in aged, somatic cells. They even accumulate in the germ line. One experiment with frozen semen collected from the same bull at young and old ages (Hill *et al.*, 2000a) suggested that the quality of embryos produced with this semen declined with age of the bull at the time the semen was frozen. Perhaps the most striking example of a mutation affecting phenotype of genetically identical individuals is human monozygotic twins of opposite sex (Wachtel *et al.*, 2001).

There are two separate issues with mutations: (1) different genotypes among clonal donor cells and (2) mutations in organisms postcloning. Both will result in differing genotypes and, often, phenotypes among clonal sets. An intriguing ques-

tion concerns what can be done to minimize genetic differences among clones due to mutations. This is obviously related to questions of aging (Seidel, 2000). Very likely, there will be fewer mutations in cells of some tissues than in others. Germ line cells may have more mechanisms to minimize mutations compared to somatic cells (Seidel, 2000). Cells that are constantly dividing, such as hematopoietic cells, probably accumulate more mutations than do those that rarely divide, such as Sertoli cells. Granulosa and cumulus cells in ovarian follicles arise from follicle cells that have been quiescent since fetal life, and have been dividing for only a few months prior to ovulation. They may have dual advantages of fewer mutations and being more readily reprogrammed compared to other cells. A final, obvious tactic is to use younger rather than older donor cells; with planning, young cells from animal cloning candidates can be cryopreserved for cloning at a later date.

## CULTURAL INHERITANCE

Although largely anecdotal, there also is solid evidence that some learned behavioral traits are passed on from generation to generation in various animal species, generally through the maternal line (Avital and Jablonka, 2000). This cultural transmission is easily broken with embryo transfer to surrogate recipients, thus imposing a different maternal learning paradigm. An example is cattle grazing extensively in the semiarid foothills and mountains of western North America. Cows have a choice of dozens of microenvironments over thousands of acres, and cows seem to teach their calves where and what to graze, or even browse brush in some cases. This is passed on from year to year maternally (essentially all males are sold for fattening). One practical consequence is simply finding the cattle at round-up time each fall; searches on horseback for hundreds of cattle occur over hundreds of square kilometers, yet certain cows and their progeny and grand progeny can be found, year after year, hidden in the same areas. To the extent that such phenomena occur, the sorts of phenotypes described obviously will not be the same within sets of clones unless the surrogate recipients come from the same maternal pod.

## HOW SIMILAR?

All sorts of mechanisms have been described that can cause genetic, epigenetic, and phenotypic differences within clonal sets. However, just how much variability will there be for practical purposes? This obviously will depend on the environment, the species, and the trait or traits of interest. We already have huge amounts of information to answer such questions from inbred strains of animals and their  $F_1$  crosses, and from identical multiplets as well as from clonal sets to a limited degree (Green *et al.*, 1996; Gärtner *et al.*, 1998). Genetically identical rodents living in standardized, noncrowded environments are very similar in traits such as body weight at given younger ages. However, as they age, large differences occur in various traits, particularly longevity (Finch and Kirwood, 2000). An example of practical behavioral variability is provided by the multiple cages of male rodents kept for purposes such as breeding superovulated females. Some males are better performers than others, even though they are of the same strain and age, managed as identically as practicable.

The extreme of natural variability among clonal sets is human identical twins reared apart. Although a discussion of this literature is beyond the scope of this chapter, there is considerable phenotypic variability between such individuals, as well as among identical twins reared together (Bouchard *et al.*, 1990).

Between the two extremes just described is where most cloning applications fall, and where genetic and phenotypic differences and similarities of clones are most relevant. The major immediate application of cloning by nuclear transfer is research, such as studies on reprogramming DNA. This special case is discussed in detail else-

where in this volume. The nonresearch applications, other than to replace defective human organs and tissues, and production of transgenic animals to produce pharmacological products, seem to fall into three broad categories: replacing cherished companion animals, copying valuable athletic animals, and producing agricultural animals. Businesses already exist to address these opportunities. The first cloned kitten, announced by a group in Texas, has been produced to promote a commercial endeavor.

Although cloning a pet or a valuable racing camel will not result in the same animal, such animals will certainly be very similar to the donor in many phenotypic respects. Most pet owners likely will be quite satisfied with the product, even though there likely would be clearly measurable phenotypic differences between the donor and the clone. If the animal is of a spotted strain, the spots will not be identical, but otherwise will probably be sufficiently similar to satisfy the customer in most cases. The racing camel owner is, however, likely to be disappointed if the objective is racing ability. There likely will be a considerable regression toward the mean for traits such as athletic ability. Such traits, of course, have huge environmental components, which can be controlled to a considerable extent. However, there also are likely to be substantial epigenetic effects that are impossible to control. If a prized animal were being copied for breeding purposes because of being a proved sire, then success is probable because a genetic trait is the objective. Cloning provides the opportunity for several classes of sterile animals to reproduce or be reproduced. An intact copy of a castrated male can be made; this might be especially relevant for gelded horses. A second example is mules, hinneys, and the like.

Perhaps animal agriculture is considered by most people as the obvious, most legitimate large-scale application of cloning technology. Use of this technology for improving meat and milk production has been considered carefully by Smith (1989) and Van Vleck (1999), among others. The numerous studies on identical twin cattle also provide information about the maximal degree of identity that is possible in agricultural production traits. Basically there are two fundamental questions for quantitative agricultural traits: (1) Accuracy—to what extent is an animal identified as truly phenotypically superior and, in turn, genetically superior? (2) Heritability—to what extent is the superior phenotype due to genetics on the average and, therefore, how superior will the average clone be?

At least for bulls, when they have thousands of offspring, it is already possible to know genetic superiority for certain traits with up to 99% accuracy. It will also be possible eventually to determine the true superiority of any individual by making large clonal sets. However, in the absence of such tests, the true genetic value of an individual superior animal will only be estimated imprecisely for most traits (Van Vleck, 1999). This means that selecting an animal with an outstanding phenotype for a trait, such as milk production, and cloning it will often lead to disappointment, simply because this animal was not truly superior genetically. As mentioned earlier, clones of animals with extreme phenotypes, for example 3 or more standard deviations from the mean, likely will not live up to expectations. On the average, such clonal sets will be superior, but not as superior as expected (Van Vleck, 1999), and some will not even be superior to the mean. Thus, a testing step costing considerable time (around one generation) will be needed in most cases to determine true phenotypic superiority of a clonal set. To the extent that there is available genetic information on relatives of the superior animal in question, one can bolster confidence that this animal is truly superior. An extreme example is the bull with recorded information on thousands of offspring. Often techniques such as marker-assisted selection and genotyping for specific alleles also can be used to improve chances that the animal being cloned is good starting material.

The second important aspect of cloning from an agricultural production standpoint is similarity among members of clonal sets, which is governed by the relationship between genotype and phenotype, or heritability (abbreviated  $H^2$  for the

sum of all modes of inheritance). For some traits  $H^2$  is virtually 100% (e.g., coat color) and for others  $H^2$  is close to 0% (e.g., fertility). For most important agricultural traits, such as growth rates, milk production, meat or milk composition, or docility,  $H^2$  usually is on the order of 20–50%. This has two consequences. First, the clone mates of a truly phenotypically superior animal (e.g., a cow producing 14,000 kg of milk/year in a herd with an average milk production of 10,000 kg) will average only 11,000 kg of milk/year if  $H^2$  is 25%. Second, there will be huge variation from clone to clone. Note that increasing average milk production from 10,000 to 11,000 kg in one step is a huge gain relative to other breeding techniques. However, it will not be the 4000 kg gain that many people expect. The situation is better than that just described to the extent that environments are standardized because of genotype  $\times$  environment interactions. Thus, clone mates on the same farm or among farms where the environment is exceedingly similar will be somewhat more similar to the original animal and to each other than an  $H^2$  of 25% predicts. The bottom line, however, is that genetic principles still operate with cloning, that cloning does not copy phenotypes, and that, for many traits, most phenotypic variation is not due to genetic variation.

## SUMMARY AND PERSPECTIVE

Members of clonal sets of mammals produced by nuclear transplantation will be genetically identical except for mutations and mitochondrial genes (if the same maternal line is not used for donor nucleus and recipient cytoplasm). Numerous chance epigenetic phenomena, some of which are mediated via differential methylation of cytosines, will affect members of the same clonal set differently. Superimposed on these effects are uterine effects and other environmental vagaries postbirth, even under relatively controlled conditions. For some species, situations, and traits, there will only be small phenotypic differences among members of cloned sets. For other species, situations, and traits, phenotypic variation will be fairly large.

For many applications of cloning by nuclear transplantation/cell fusion, the phenotypic variation among clones will be of minimal consequence. For other applications, the phenotypic variation within clonal sets may negate most of the potential benefits of the procedure. Cloning by nuclear transplantation is an exceedingly valuable tool; however, it is a tool, not a panacea, and it never can recreate the same animal.

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