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Precision animal breeding

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We accept that we are responsible for the quality of life of animals in our care. We accept that the activities of man affect all the living things with which we share this planet. But we are slow to realize that as a result we have a duty of care for all living things. That duty extends to the breeding of animals for which we are responsible. When animals are bred by man for a purpose, the aim should be to meet certain goals: to improve the precision with which breeding outcomes can be predicted; to avoid the introduction and advance of characteristics deleterious to well-being; and to manage genetic resources and diversity between and within populations as set out in the Convention on Biological Diversity. These goals are summed up in the phrase precision animal breeding. They should apply whether animals are bred as sources of usable products or services for medical or scientific research, for aesthetic or cultural considerations, or as pets. Modern molecular and quantitative genetics and advances in reproductive physiology provide the tools with which these goals can be met.

Keywords: animal breeding; molecular genetics, application of; quantitative genetics, application of; reproductive technologies, application of; responsibility for breeding outcomes

1. INTRODUCTION

We breed animals for four principal reasons: as sources of usable products or services; for medical or scientific research; for aesthetic, cultural or ethical considerations; and as pets. The first leads to animal husbandry and livestock breeds of domesticated species kept for food, fibre and other services such as transport and power; the second provides laboratory animals of defined genetic lines, including animals with gene knockouts; the third encompasses breeding for conservation; and the fourth leads to companion animals used for pleasure or recreation. Objectives of sustainability should apply in each case.

Precision animal breeding should be used whenever animals are bred *for a purpose*; that is when they are bred for a particular use, environment or market. It should therefore apply when animals are bred for any of the four reasons described above. This is appropriate as the techniques used are largely generic. Our aims in this paper are: (i) to review the methods currently available, (ii) to indicate how they have developed over time to become more precise, (iii) importantly, to show how generic technologies are giving opportunities to substantially improve precision, and (iv) to show what needs to be done to deliver these opportunities. In many cases, the breeding of animals is controlled or influenced by legislation, or by national and international bodies. It is not possible to review precision animal breeding without reference to either of these factors, or the sizes of animal populations or their economic and cultural significance, and for this reason it has been necessary to set the science within a wider background. On the other hand, we have had to limit the range of species considered and will therefore concentrate on terrestrial vertebrates.

The twentieth century marked a turning point in our relationship with other species from which there is no way back. The human population explosion led to widespread competition with other species for agricultural land, and many species became extinct or are now threatened with extinction primarily as a consequence of these activities. We also began to recognize how our industrial culture influences our environment not only locally but also at a distance through atmospheric pollution (e.g. acid rain), and globally through climate change.

We accept that where animals are already within our care, we are responsible for their quality of life. We accept that the activities of man affect all the living things with which we share this planet. But we are slow to realize that as a result we have a duty to care for all living things. This duty extends to the breeding of animals for which we are responsible.

It is in this context that we propose that precision animal breeding should set the following goals.

— To increase the scope and precision of predictions of the outcomes of breeding decisions (G1).
— To avoid the introduction and advance of characteristics deleterious to animal well-being or, more generally, the well-being of the species (G2).
— To manage genetic resources and diversity between and within populations in accordance with the principles set out in the Convention on Biological Diversity (http://www.biodiv.org) (G3).

While these goals will be discussed in more detail later, we shall advance some initial arguments for their form. The first of these goals is clear in the context of precision breeding! However, we have the potential

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through genomic technology to be more precise in directing the outcomes to be closer to what we desire, both directly in what we select for and indirectly in reducing the chance of unforeseen consequences. The second goal recognizes not only our responsibility for quality of life in populations that are managed, but also some of the ethical conflicts that arise in conservation activities, where an aim for a captive population is to support or re-establish a wild population, and where domestication through adaptation to captivity may be harmful. The third goal recognizes the force of the arguments clearly articulated in the Convention on Biological Diversity, which apply to all our genetic resources both managed and unmanaged.

To meet these goals for precision animal breeding there is a requirement to establish:

— breeding objectives in the context of the environment in which the progeny are to be kept (R1);
— a robust parameterized model of the extent of genetic variation, for example heritabilities of traits selected and genetic correlations among significant traits, together with estimates of breeding values (BVs) for significant traits for individual animals (R2);
— an understanding of gene expression from genotype to phenotype, including the molecular basis of traits (R3).

In practice, for the vast majority of species all this information is seldom available. At best we meet R1 and R2 and a small component of R3 in a limited number of commercially valuable populations. It should be an urgent goal to extend that number as rapidly as possible in order to meet the goals of precision animal breeding, as once desirable population characteristics are lost or conversely deleterious characteristics are introduced, the desired position can be slow and costly to retrieve. Furthermore, in view of the international trade in genetic resources, this need for information must be considered at an international level.

There is a continuum between the genetic structures of small, endangered populations and those of widely used livestock species, and in many cases the same issues affect both. For instance, both the Holstein dairy cow and the Arabian oryx (*Oryx leucoryx*) are affected by concerns over rates of inbreeding. In the case of the dairy cow this results from the widespread use of a limited number of desirable bulls through artificial insemination. However, the degree of inbreeding in these two species differs markedly: for the dairy cow, $F \approx 2.5\%$ in the UK to a 1950 base (Kearney *et al.* 2004) whereas for the oryx in 1985 $F \approx 60\%$ to a 1970 base (Mace 1988). Nevertheless, the methods used to trace an animal's evolutionary history may be applied to both rare and widely bred species: for instance microsatellite markers have been used to understand the evolutionary history and current genetic status of Arabian oryx (Marshall *et al.* 1999), South American camelids (Bustamente *et al.* 2003) and the Jersey cow (Chikhi *et al.* 2004). The same procedures are therefore available for application to all managed species, in both small and large populations. Whether it is appropriate to apply them depends on the question being asked and the cost-effectiveness of the procedure, given that there may be other calls on resources.

2. THE STATE OF THE ART

The state of the art of animal breeding is based on integrating aspects of many sciences and technologies, with the degree of sophistication varying considerably between the different areas of interest and activities. The key scientific inputs range from genetics (both quantitative and molecular), statistics and computing science, information technology, and the physiology and endocrinology underlying reproduction and fertility. The integration concerns the objectives of the breeding, testing, recording, evaluation, selection and mating of breeding animals.

The objectives of the different areas of breeding activity vary widely. In livestock, well-defined objectives (R1) are necessary for success and will usually evolve over time in relation to societal concerns. Objectives have been for productivity with higher output per unit input but, while these objectives are still important, there has been a notable shift towards tackling issues related to reproduction, well-being and longevity. The ability to tackle the latter objectives is currently constrained in many parts of the world, including the UK, by a combination of poor information on these traits owing to the lack of routine recording coupled to the poor genetic 'signal' within this information (measured by the heritability; see below). In conservation activities the objective is primarily to increase population size and conserve genetic variation; however, where conservation management includes breeding for release, an important genotype-by-environment interaction arises since breeding in captivity must address the objectives of non-domestication and fitness for wild environments (Gilligan & Frankham 2003).

To illustrate the state of the art we shall describe dairy breeding as a reference and indicate how other sectors differ in technology and operation. The challenge with dairy breeding is that key traits, such as milk production, are sex- and age-limited, and this determines much of the structure of the industry. These sex- and age-limited traits can only be measured in the mature female, reducing the scope for selecting animals on their own performance and the speed at which selection can occur. This is particularly restrictive as it is the male that has the much higher reproductive rate, particularly since advances in reproductive sciences led to the introduction of artificial insemination some 50 years ago. Therefore, improvement systems have developed that are based upon progeny testing in which the genetic merit of candidate bulls is judged by a large number of daughters (100 or more in some cases) being milked on many private farms across the country. Conducting such a test efficiently is a challenge requiring efficient statistical design, the routine gathering of records on performance, and their collation and storage in databases for evaluation.

The estimation of genetic values for the bulls being tested, and for all other animals in the dairy herd, is made using these data and all the other records

collected on dairy cows, using best linear unbiased prediction (BLUP; see below). This statistical technique is based upon detailed genetic models (R2) and accounts for the diverse environments of the thousands of different farms from which the data has been collected. The computational challenge is considerable: predicting BVs for millions of animals across thousands of farms, for hundreds of traits, requires major computational and statistical efforts.

Agricultural breeding companies in the pig and poultry sectors are similarly developed operationally, although less reliant on progeny testing, and with the added benefits of the ability to manage the necessary size of population and the scale of recording from within their own resources. Additionally, several breeding companies use sophisticated selection techniques and mating procedures to minimize the loss of genetic variation in the process of generating improvement (Woolliams *et al.* 2002), a clear step to achieving G3 within commercial livestock. For some sectors, a limited number of genetic markers may contribute information to the selection process; where this is done the most valued markers are those that address traits such as disease or reproduction where other genetic information usually has poor accuracy. Other sectors such as sheep breeding are less well developed in this respect.

For companion animals and equids, the state of the art for breeding is centred upon the use of pedigree records and shows to exhibit examples of the breed and identify desirable parents, together with the occasional use of reproductive technology to increase the reproductive rates of such parents. In equids, there is a move internationally towards more reliable statistical evaluations based on performance and formal testing of young horses.

Animals bred for science differ again. The populations of mice that are used are predominantly well-documented inbred strains and crosses derived from them. The use of transgenic technology and mutagenesis to produce novel phenotypes has increased significantly. Limiting the range of species considered here precludes mention of *Drosophila*, which continues to make a huge contribution to functional genomics, and zebrafish.

(a) *Population genetics*

(i) *Heredity and quantitative traits*

The physical and behavioural characteristics of organisms are determined by the genes they inherit and the environment in which they develop. In terms of physical characteristics, this is widely accepted. Those doubting the importance of genetics in controlling behaviour should consider that behaviours are determined by physical characteristics of neuronal pathways and interactions (e.g. Kendler 2003).

There are approximately 30 000 genes coding for proteins in the nuclear genome of the human being (Southan 2004), which may be taken as a working estimate for other vertebrates. Genes are composed of sequences of nucleotides, and there are differences between individuals in the sequences of nucleotides within a gene known as *polymorphisms*. There are also differences between individuals in the degree to which genes are expressed due to polymorphisms in the regions controlling transcription or in other genes, which control transcription in *trans*. These differences between individuals (and others described under *epigenetics*; see below) account for the genetic differences between individuals, which together comprise the *genetic variation* in a population. Where genes determine quantitatively measurable characteristics, these characteristics are known as *quantitative traits*.

During fertilization, an individual receives one copy of each gene (with a caveat over sex-linked genes) in the gamete received from each parent, with the genes passed in discrete blocks called chromosomes. In humans, the number of chromosomes passed from parent to offspring is 23, and in cattle 30. The chromosomes passed by the parent will be a random choice among the pair of homologous chromosomes carried by the parent made during the process of gamete formation, called meiosis. However, the process of meiosis involves *recombination* in which segments of the two homologous chromosomes carried by the parent are exchanged. As a result, new gene sequences may arise. However, there are few recombination events relative to the total number of genes. In addition, variation can arise from new mutations within an individual that change a nucleotide or involve more complex deletions and rearrangements, and change the functional properties of a gene. If these occur within cells of the germ line, these mutations can enter the gene pool of the population and add to the genetic variation observed.

What is observed is the phenotype, which is determined by the sum of the genetic effects and the environmental effects. The latter is the accumulated impact of events in the life history of an individual and, possibly, its parents or even more distant ancestors (e.g. Benton *et al.* 2005). The primary clue for recognizing the existence of genetic variation is the finding of a degree of resemblance between relatives, especially when their development has occurred in different environments.

While we can observe genetic variation within a population, not all of it can be used to produce a change in the population mean; the portion that can be used in this way is called the *additive genetic variation*. It is knowledge of the extent of variation and its different portions that comprise a requirement R2 for precision animal breeding. Knowledge of the precise mutations that underlie genetic variation is unnecessary in order to achieve improvement. For example, despite the doubling of milk yield since 1940 and the many genes that affect milk yield, no single mutation having a direct and verifiable effect on yield was known until 2000 (Andersson & Georges 2004). The better our understanding of the variation, advancing to knowledge of the important mutations underlying variation, i.e. including addressing requirement R3, the more capable we will be of achieving the goals G1 and G2 of precision animal breeding.

The superiority of the offspring from an individual parent when compared with the population mean leads to the concept of the *BV*, which is a measure of the additive component of the parent's genes and its suitability for *selection*. Most quantitative traits are

determined by many genes acting in concert (i.e. are *polygenic*), and so an individual's BV cannot be considered the property of a single gene, but of many genes in combination. Breeding schemes are a long way from achieving full precision so man-made selection (*artificial* rather than natural selection) is made on an *estimated breeding value*.

(ii) *Genetic correlations*

Since some genes affect more than one trait, and since some genes, being located close to one another on the same chromosome, tend to be inherited together, there are *genetic correlations* between certain traits. That is, genetic improvement through selection for one trait may lead to a change (improvement or otherwise) in another. This has been recognized since selective breeding began; Darwin, in *The origin of species by means of natural selection* (chapter 1) states:

> Hairless dogs have imperfect teeth; long-haired and course-haired animals are apt to have, as is asserted, long or many horns; pigeons with feathered feet have skin between their outer toes; pigeons with short beaks have small feet, and those with long beaks large feet. Hence if man goes on selecting, and thus augmenting, any peculiarity, he will almost certainly modify unintentionally other parts of the structure, owing to the mysterious laws of correlation.

Genetic correlations have resulted in undesirable and unexpected side effects of selection in many species. In dairy cattle for instance, selection for yield has resulted in progressive loss of fertility, so that while yield has increased annually in the UK by approximately 90 kg per lactation for the past 20 years, rates of fertility (measured as per cent of animals conceiving to first service) have declined during this period by about 1% per annum (Royal *et al.* 2000): the genetic correlation between yield and calving interval in UK Holsteins is 0.27, and between yield and days to first service, 0.67 (Wall *et al.* 2003). To counter this effect, *fertility indexes* have been introduced initially in Nordic countries and more recently in the UK (Flint *et al.* 2004) to promote those bulls with high yielding, fertile daughters which, though fewer in number, are present in the population. Examples of other genetic correlations that have adversely affected breeding outcomes include the correlation of growth with egg production in poultry (Pirchner 1986) and fleece weight with fertility in sheep (Fogarty 1995). It is only by understanding the mechanisms that lead to genetic correlation, through advances in knowledge of gene expression, that we can be truly proactive in achieving goals G1 and G2, rather than only responding to what is observed.

(b) *Molecular genetics, genome sequencing*

The revolution in molecular genetics, and particularly genome sequencing, has already provided benefits for animal breeding, but in comparison with what the future holds, our present tools will undoubtedly be seen as primitive. At the time of writing, complete genome sequences are available for a number of species, genome sequences for the chicken, cow, horse, mouse and chimpanzee are either completed or nearing completion, and single nucleotide polymorphism (SNP) libraries for these species are growing rapidly. This information will underpin most of the developments in livestock breeding and breed management during the coming two decades (see §3*a*(i) on the structure of the industry).

(i) *Molecular markers, microsatellites and single nucleotide polymorphisms*

The value of molecular information in assisting breeding decisions has already been demonstrated, in particular through the use of marker-assisted selection as well as for monitoring population structure, and for information on the history and development of populations. Early examples of gene discoveries in livestock that were subsequently used for marker-assisted selection were: a mutation causing malignant hyperthermia in pigs (Fujii *et al.* 1991); a microsatellite closely linked to the mutation causing weaver disease in cattle (Georges *et al.* 1993); a marker for increased litter size in pigs (Rothschild *et al.* 1996); and a deletion leading to double muscling in cattle (Grobet *et al.* 1997). The use of DNA markers for monitoring intra-breed structure has been verifiably illustrated by Chikhi *et al.* (2004) in their study of Jersey cattle on the Isle of Jersey. An example of use for inter-breed structure is given by Wiener *et al.* (2004).

The primary route of development for DNA marker technology has been from restriction fragment length polymorphisms (RFLPs; which arise from loss or gain of sites where genomic DNA is cut by restriction enzymes), through microsatellites (arising from variation in the lengths of repeated sequences of satellite DNA) to SNPs (single point variations in nucleotide sequence). Among other marker types, SNPs share the benefits of being: (i) widespread throughout the genome and so close to coding regions, (ii) co-dominant hence heterozygotes can be identified, and (iii) amenable to the use of PCR to amplify the signal. The use of microsatellites has been widespread over the last decade, with advantages over RFLPs in the multiplicity of alleles at a single locus and a greater repeatability, although reproducibility across laboratories is still a problem. The technology surrounding SNPs has recently improved by orders of magnitude, so that assays can be scaled and automated, resulting in a low cost that outweighs the disadvantage of being predominantly biallelic. Blott *et al.* (2003) estimated that it is necessary to have five or six closely linked SNPs to replace the information contributed by a typical microsatellite, but given the current approximately 100-fold difference in cost per genotype, the benefit is clear. One outcome of such a reduction in cost is that a cattle genome can be densely marked with 50 000 SNPs at a current (2007) cost of approximately £200; this is a small cost when compared with the cost of breeding and testing a dairy bull for example.

Changes in nucleotide sequences within coding regions are termed 'silent' if they make no change to the amino acid coded, but if silent they may change transcription rates or transcript turnover, thereby affecting protein levels. However, the complexity of control of DNA transcription is still poorly understood. An example of this complexity arising from a single

SNP is the polar overdominance in the ovine callipyge gene (Freking *et al.* 2002).

(ii) *Mitochondrial DNA*

Mammalian mitochondria contain a small circular DNA plasmid of 16.5 kb which codes for 37 genes required to be expressed within the inner mitochondrial membrane. The mitochondrial genome evolves 17 times faster than nuclear DNA, probably due to lack of DNA repair mechanisms. As a result, the mitochondrial DNA sequence can be used to monitor evolution on a shorter time scale than is possible with chromosomal DNA. Mitochondrial genome polymorphisms are therefore frequently used to analyse population structure and demographic history. Mitochondrial DNA is haploid, and so each individual has a single haplotype. It is maternally inherited as a result of the limited contribution to the zygote of mitochondria from sperm. This limits its use in relation to domestic species where gene flow through the male line forms an important determinant of evolution and population structure, for instance through artificial insemination, but provides the advantage that it allows introgression through the female line to be distinguished from that through the male.

The value of mitochondrial DNA for animal breeding is that it allows an understanding of population history and structure in time and space. For instance, a low level of mitochondrial DNA polymorphism within a species suggests it has survived a reduction in population size or bottleneck, whereas a high level of variation is characteristic of a large and well-established population. Mitochondrial DNA is extremely valuable in resolving important taxonomic questions when distinguishing subspecies and in identifying evolutionary significant units (ESUs; see §5*c*). Mitochondrial DNA bar coding has been suggested as an aid to assessment of biodiversity (Hebert *et al.* 2003), but this has been questioned, particularly in species subject to parasite infestation or with a high incidence of symbiont infection (e.g. arthropods; Hurst & Jiggins 2005). The general conclusion from large-scale analyses of livestock populations, which are perhaps the most informative for this purpose, suggests that variation in maternal lineages explain at most a small fraction of the variation in traits of commercial interest (e.g. Roughsedge *et al.* 2000*a*,*b*).

(iii) *Epigenetic effects*

Certain heritable characteristics are encoded in DNA by covalent modifications to chromosome structure rather than the nucleotide base sequence of genes. Examples of these modifications are the acetylation of histones in nucleosomes, which alters the ability of the transcriptional enzymes to open and copy the DNA, and the methylation of cytosine residues in DNA, altering their protein-binding characteristics. These alterations in chromosome structure are responsible for genome imprinting, which determines whether a copy of a gene received from the mother or father is transcribed. This process is responsible for genetic diseases such as Huntingdon's chorea through revealing a deleterious mutation that remains uncompensated. Imprinted genes are also involved in the expression of the callipyge mutation (Freking *et al.* 2002) in sheep.

(c) *Genetic evaluation*

By knowing BVs, changes can be made in traits without knowing what genes are responsible, and this is what has been exploited throughout domestication: the heritability of characteristics and the similarity of relatives. The development of this process has produced the advanced methods available today, of which BLUP is the epitome for the present.

The introduction of accurate methods for the estimation of BVs has been one of the major successes in precision animal breeding. The need for improved statistical techniques was recognized following the development of artificial insemination during the 1940s, and the introduction of progeny testing for dairy bulls in the 1950s. The method used at that time was *contemporary comparison*, which used production data on a bull's daughters during their first lactation, compared with other cows in the same herd during the same year and season. Effects were then combined across herds.

Contemporary comparison recognized the importance of environmental effects, but failed to take into account differences between herds in genetic merit. It also assumed that all animals other than the daughters of the sire under test were unrelated, and this became increasingly invalid following introduction of artificial insemination. To overcome these difficulties, a method was required which allowed for a wider variety of environmental variables, and took account of the genetic relationships between the animals sampled. These requirements were met by the introduction of BLUP developed by C. R. Henderson in 1949 (Henderson 1975). This statistical method uses matrix algebra to solve the large numbers of simultaneous equations generated for estimating BVs and for identifying environmental effects simultaneously. Implementation of BLUP required considerable computing power to solve the matrices generated. In particular, the complexity of the genetic information used dictates the amount of computer space or time required. In dairy cattle breeding, sire model BLUP evaluations were first used in the US in the early 1970s, sire–maternal grandsire BLUP evaluations were introduced in the UK in 1979 and animal model BLUPs in 1992. BLUP is the current method of choice for evaluation in all sectors and is widely implemented.

(d) *Reproductive technologies*

The development of population genetics methodologies and their application to livestock breeding coincided with the development of reproductive physiology and the introduction of reproductive technologies during the last half of the twentieth century. These technologies include artificial insemination and embryo transfer, both of which have been used extensively in the international dissemination of genetic resources. The development of assisted reproduction techniques in human medicine has gone hand-in-hand with their application in animal breeding, advances in human medicine being dependent on studies in animal models.

(i) *Artificial insemination and multiple ovulation and embryo transfer*

Examples of the impact of reproductive technologies on the dissemination of improved genetics are to be found in the turkey and dairy breeding industries. Commercial turkey breeding is now dependent on artificial insemination, because the large size of the males of broad-breasted turkeys, which have been bred for body conformation, precludes natural mating (a failure to apply precision animal breeding). However, artificial insemination provides great benefits. Without semen cryopreservation and artificial insemination the dairy industry would not have developed in the way that it has, since the 1950s, through the international trade in semen and embryos, and these techniques have also had an impact, though less dramatically, on the beef and sheep industries. Since these methods were developed, we now have semen and embryo sexing and multiple ovulation and embryo transfer (MOET) technologies, somatic nuclear transfer and assisted reproduction techniques developed in human medicine, such as intra-cytoplasmic sperm injection. All these techniques, but particularly semen cryopreservation and artificial insemination, play a major role in the design of breeding programmes and in the dissemination of advanced genetics, although the use of nuclear transfer is limited at present. These technologies allow better identification of merit and increased selection intensity through increasing reproductive rate and better use of resources for testing.

The benefits of artificial insemination do not require its widespread use. For instance, artificial insemination makes *sire referencing schemes* possible, where a number of farms cooperate in a breeding programme, using the same reference sires. This has the advantage that by using a small number of semen donors, accurate genetic comparisons across farms can be obtained. These schemes have been extensively used in breeding sheep and beef cattle, and have also been used in the UK for red deer.

(ii) *Transgenesis*

Use of gene insertion and gene knockout techniques has been largely limited to the production of animals for medical research (see §4*a*). An exception has been in the production of animals secreting proteins of pharmaceutical value in their milk (Colman 1996). The artificial modification of genes encompasses transfer of genes from one species to another, or altering the genome to remove or suppress the expression of existing genes. Within livestock breeding there are no transgenes within the gene pools of commercial populations at present, outside the experimental stations of various countries. In some countries, for example Norway, the use of transgenesis in livestock is prohibited on principle. What is clear is that the difficulties of ensuring the necessary integration of the transgene into the genome (both in controlling expression of the transgene and integrating expression with the remainder of the genome) has been a challenge of questionable cost–benefit. Early efforts in this area (e.g. Wagner *et al.* 1983) clearly failed in relation to principle G2 that we advocate for precision animal breeding. However, future advances in understanding may lead to applications that satisfy all the principles listed and which will have a benefit sufficiently large to carry public opinion, for example in the prevention of endemic diseases and zoonoses.

(iii) *Nuclear transfer and cloning*

Besides its potential to contribute to the study of developmental biology, somatic nuclear transfer has two practical advantages: as a route to transgenic animals and stems cells, and as a means of conserving nuclear genomic material. Cloning by nuclear transfer has now been applied in many species: sheep (Dolly, the first from an adult cell; Wilmut *et al.* 1997); goats; cows; pigs; horses; a rhesus monkey; rats; mice; dogs; cats; rabbits; and gaur.

As a means of producing transgenic animals, the advantage of nuclear transfer lies in the modification and selection of nuclear donor cells in culture, before transfer of their nuclei into enucleated oocytes. Following transfer, factors in the oocyte cytoplasm reprogramme the donor nucleus as a result of which it reverts to totipotency. In this respect, the technique provides the advantages of embryonic stem (ES) cells in species other than mice, which is important because stem cells have not been derived for domestic species. All the gene transfer methods applied to mouse stem cells can be used in cells for nuclear transfer, including random gene insertion, targeted gene replacement by homologous recombination and targeted gene insertion. Selection after transfection can be by toxin resistance, and changes can be monitored by standard molecular biology techniques before cells are used. Despite some improvements in the success rate of nuclear transfer (Campbell *et al.* 2005; Lee & Campbell 2006), much still needs to be done to understand the limitations inherent in the process, and to develop more efficient procedures.

The advantage of nuclear transfer as a means to conservation lies both in the ability to freeze somatic cells and in its use to rapidly increase population sizes. Somatic cells are easier to obtain than gametes, although freezing/thawing regimes are better developed for sperm and eggs in a number of species than for other cells. Once transferred to oocytes, the population of cells represented by the conserved culture is rapidly promulgated. For instance, in cats, large litters can be obtained from the normal mating of individuals, both of whom were produced by nuclear transfer, and this methodology is applicable to rare species such as the African wild cat (Gómez *et al.* 2004).

Applications of nuclear transfer to agriculture include not only the preservation of valuable individuals and rare breeds, but also the rapid propagation of high-genetic-merit animals and animals of specific genotypes (Woolliams & Wilmut 1989). In this respect, nuclear transfer will benefit precision animal breeding by increasing the predictability of livestock performance (G1) and, perhaps paradoxically, fulfilling G3, although Woolliams and Wilmut highlight the risks associated with reducing local diversity through the use of nuclear transfer.

3. ANIMALS FOR FOOD AND OTHER PRODUCTS

(a) *Value added by selective breeding*

A conservative estimate of the annual value of livestock production in Europe is €123 billion, and the annual genetic gain at the level of the producer is equivalent to 1.5% of that figure, i.e. €1.8 billion (FABRE-TP 2005). The annual research and development costs of breeding organizations, including collecting data for estimating BVs and carrying out breeding programmes but not product marketing, is approximately €150 million, a benefit to cost ratio of 10. Genetic gains are permanent and cumulative so that the gain made in one year will give benefits over all subsequent years without further intervention, and this increases the benefit to cost ratio cohort by cohort. This contrasts with vaccination strategies, for example, where the benefit from a vaccine requires repeated application, cohort by cohort. The livestock sector in Europe employs 3.5 million people and remains the largest sector in agriculture in terms of both employment and output value. Therefore, genetic progress is central to the success of this major industry.

(i) *Structure of the industry*

Progress is principally in the hands of a small number of large institutions. The costs associated with collecting information on economically significant traits, developing and maintaining extensive pedigree and other databases, using up-to-date statistical techniques and large computer systems are high, and as a result much of the expertise is concentrated in a few large commercial companies. For example, over 90% of global poultry breeding stock (layers, broilers and turkeys) is in the hands of two or three organizations selling to worldwide markets in each case. Similar situations apply for pigs and dairy cattle. On the other hand, there are a large number of small breeding organizations dealing with individual breeds, which are unable to benefit easily from the advances open to larger companies. This barrier can be overcome through cooperatives, and effective examples can be found in many European countries (e.g. the Genesis Faraday Partnership scheme in the UK).

(ii) *Aims for genetics within livestock industries*

The general aim of the livestock sector is to meet the aspirations of the world's population for increased availability of animal products in a sustainable manner while ensuring food safety, animal welfare and the maintenance of rare and specialist breeds. The expected worldwide increase in consumption of animal products for the next decade is 7% annually. The availability of marginal land suitable for producing this increase is limited, and hence there is a need to produce more from the same resources (Food and Agriculture Organization 2000). For genetics to contribute half the required acceleration would mean a doubling in genetic gain (from the figure of 1.5%; see above). This illustrates both the need and the opportunity for livestock breeding, and placed alongside the requirements for sustainability, the need for precision animal breeding. Sustainability in livestock production implies meeting production targets while ensuring targets are also met for environmentally significant outputs, human feed efficiency, animal health and welfare and maintenance of biodiversity, in both farmed livestock and wild species affected by animal husbandry.

Modern agricultural practices often reduce, rather than increase, genetic diversity in domestic animal populations. Selection for desirable traits and the rapid dissemination of genetic material through populations by artificial insemination and embryo transfer, including international trading in genetic material, all tend to reduce genetic diversity. Furthermore, genetic diversity is lost through the reduction of population sizes in rare breeds, or in some cases through the loss of the breeds themselves. The genetic diversity being lost in this way is not fully characterised. Such trends are contrary to goal G3 and are explored further below. Within breeding schemes goal G3 can be addressed using selection procedures that explicitly manage genetic variation whilst maximising gain through controlling the rate of inbreeding (e.g. Meuwissen 1997).

(b) *'Pharming' and xenotransplantation*

Most pharmaceuticals currently available are small molecules. Their chemistry is accessible and they are relatively cheap to produce. In contrast, many therapeutic compounds are proteins, and advances in functional genomics and proteomics will identify many more. Proteins are, however, costly to produce at the purity required for clinical use through current cell culture techniques, and it would be highly desirable to have available alternative methodologies. Secretion of proteins into milk and other biological fluids offers one such route to production of valuable proteins. For example, to bring on-stream a cell culture facility for drug production takes 4 to 5 years and costs over £100 million, whereas to produce a transgenic founder animal and develop a herd of cows derived from it would cost less than £5 million (Forsberg & Bishop 2002) and take no longer. The market for recombinant therapeutic proteins was US$12 billion in 1998, with an estimated 12% growth annually to 2006 (Jasuja 2000). For monoclonal antibodies, the market grew between 1999 and 2001 from $900 million to $3.5 billion, based on only 10 products, with a further 270 products under development.

For these reasons, several companies are in the process of developing products through either microinjection or nuclear transfer in sheep, goats, cattle and pigs. For secretion into milk the most commonly used promoters are derived from the β-lactoglobulin or α-S1-casein genes, and the products include α_2-antitrypsin, factor IX and fibrinogen. Other routes for secretion might include blood (human antibodies), urine and seminal plasma (growth hormone), each with different advantages and disadvantages: for example, one advantage of seminal plasma is the existence of the blood-testis barrier, which prevents compounds produced in the testis from acting systemically.

An alternative approach to the therapeutic use of transgenics is the modification of pig organs for transplantation into human patients. Apart from the dangers of viral transmission, this field has been slowed by the need to modify pig tissue antigens. The human immune system rejects pig tissue by an antibody response to the disaccharide galactose-α-1,3-galactose,

which is present on the cell surface in porcine tissues but absent in humans. A great deal of effort is currently being put into the development of transgenic pigs lacking this antigenic epitope, and the availability of nuclear transfer in pigs should assist in their production.

The success of these procedures relies heavily on advancing requirement R3 for precision animal breeding, an understanding of gene expression and its contribution to phenotype.

(c) *Opportunities for this century*

We are at the threshold of an era where our assumptions of what traits can be addressed by breeding, how merit is assessed and the impact breeding may have will need to be completely revised, primarily due to developments in DNA technology. Although significant strides have been made in applying the principles of precision animal breeding to domestic livestock, there are good reasons to assume that the opportunities for livestock breeding will advance much further in this century. The size of the industry associated with livestock breeding will provide a pull for technical advances, many of which will also be applicable in other sectors.

One reason for optimism is the potential for implementing *genome-wide selection* (Meuwissen *et al.* 2001). Genome-wide selection will use the dense SNP maps emerging from genome sequencing projects. The philosophy of genome-wide selection differs fundamentally from the twentieth century approach of using DNA to enable selection to use marked quantitative trait loci (QTL) or individual genes: genome-wide selection is not concerned with how many QTL there actually are, or where they are located, but rather predicts BVs after weighing uncertainties associated with each small segment of DNA. Dauntingly expensive in the past, the new SNP technologies have reduced the costs of dense genotyping in each animal so that, if not affordable now, then it soon will be. Genome-wide selection tracks gene flow and segregation in all segments of the genome and avoids many of the problems associated with QTL discovery and usage within breeding schemes that has hampered the application of DNA technology to date.

To carry out genome-wide selection, breeders will need to obtain DNA from individuals, as is done now for pedigree testing. A large number of SNPs (tens of thousands) would then be typed in each sample. This vast array of genotypes for animals would be set alongside the recorded phenotypic data on performance, and subjected to an analysis that is complex and computer-intensive, but robust, and analogous to current genetic evaluations. The potential benefits that genome-wide selection offers to livestock breeders are: (i) increased accuracy with minimal cost to inbreeding (Woolliams *et al.* 2002), (ii) ability to overcome age limitations for traits that can only be measured late in life, (iii) the opportunity to overcome or reduce sex limitations, or more generally limitations caused by measuring only special subsets, (iv) use in non-pedigree populations (e.g. identifying desirable DNA fragments in commercial populations that may be selected for within a nucleus), and (v) a direct link between the genetic evaluation and the genome. In some sectors, additional costs might be directly offset through changes in the structure of the breeding programme. These benefits would be expected to drive further innovation in both the structure and goals of breeding schemes.

To exemplify the benefits offered by genome-wide selection in the context of the aims of precision animal breeding, a goal for turkey breeding involves the improvement of growth and the maintenance of egg production. This goal is challenging owing to an antagonistic genetic correlation between these traits (Kranis *et al.* 2006); moreover, the genetic signal is stronger in growth than egg production, and the former is measurable in both the sexes, while egg production is measured only in mature females. For these reasons, traditional indices have a difficulty in making progress in growth without reducing egg production. The potential of genome-wide selection is that the genetic signal for egg production will be stronger in the young males because DNA fragments across the genome can be tracked directly from a turkey hen to her son, making it feasible to deliver the desired goals more precisely.

A further advance towards goal G1 will result from better predictive understanding of genetic correlations arising from fulfilling requirement R3. In particular, genetical genomics with its focus on understanding variation in gene expression will close a gap (the *genotype–phenotype gap*) in biology, linking variation in the genome to variation in flows through metabolic pathways, the fields of the geneticist and physiologist, respectively. Understanding the physiology mediating genetic advance will expand understanding of the physiological consequences. In turn, breeders might anticipate in what way, and to what degree, selection may have adverse consequences, and how these may be avoided.

It is therefore important to recognize that the paucity of data recorded on individual animals limits our horizons. The cliché: 'you can't manage what you do not measure' is directly relevant. Only through the accurate recording of performance and the analysis of records will the opportunities provided by these genetic advances be realized. We need to make better use of data that are already available, by establishing monitoring procedures that generate usable databases, linking these to other databases containing genetic, management or other performance information, and ensuring public databases are open for analysis by scientists. There are examples of good practice in this area such as the British Cattle Movement Service, which makes data available for specific projects. Barriers to such open availability of data need to be overcome where possible, most obviously in ensuring appropriate confidentiality and in promoting data quality when recording on farms where staff time for such activities is restricted.

High-throughput sensor technologies are also developing, mostly based on biospectroscopy, which has the potential to automate many aspects of recording, with increasing miniaturization and portability of the sensing equipment. This will include technology in milking machines for online hormone assays (see http://www.wellcow.co.uk) as indicators of fertility and disease, and for monitoring and recording in abattoirs.

Precision animal breeding has much to offer in disease control. Thirty-six farm animal diseases have a genetic basis for susceptibility (Bishop *et al.* 2003), but at present in the UK we do not monitor any of them in genetic databases. The cost of animal disease to the UK livestock industry is £1.7 billion annually, and the impact on production is 17% (between 35 and 50% in developing countries). Some (zoonotic) animal diseases are transmissible to humans. One specific and far-reaching aspiration is to integrate genome-wide selection into routine disease surveillance within the UK. Currently, Defra surveillance provides little genetic information of value, yet given the potential of the approach to dispense with the need for pedigree information, genetic surveillance may be secured by taking DNA-containing samples from casualties paired to a control sample from another animal on the same farm. This will then directly lead to a genetic strategy, delivered through genome-wide selection, coupled to more rapid and direct gene discovery arising from bridging the genotype-to-phenotype gap that is intrinsic to the approach. While the opportunity is extremely promising, research is needed to justify and quantify the benefits in order to persuade decision makers of the value of this approach.

4. ANIMALS FOR SCIENCE

(a) *Models for disease*

Spontaneous mutations, particularly in rodents, have led to important advances in functional genomics. Examples are the Brattleboro rat, which provided a model for the study of vasopressin production and function, and *ob*/*ob* (leptin-deficient) and *db*/*db* (leptin receptor-deficient) mice, which led to the discovery of leptin and its role in obesity. Since the sequencing of the human and mouse genomes, and the opportunity to understand the roles of genes in disease, the use of mutants as tools for the discovery of gene function has become more widely used. At present, the principal tools used in these studies are strains of rodents (predominantly mice) with characterized genetic mutations (http://www.informatics.jax.org/external/festing/search_form.cgi). There are in general two methods of generating genetic mutations in rodents for the development of models for investigating gene function: by chemical mutagenesis or targeted transgenesis.

(i) *Chemical mutagenesis*

Many laboratories worldwide are generating random mutations in the mouse genome by treatment with chemical mutagens such as the alkylating agent *N*-ethyl-*N*-nitrosourea. This compound causes point mutations in the double-stranded DNA of spermatogonial stem cells through mismatching to an alkylated base. Treated mice have a 1000-fold increase in the rate of mutation. The mutations occur at random in the genomic DNA, and the offspring must be screened for abnormalities in the physiological system or organ of interest. Owing to their random nature, collaborative schemes have arisen to promulgate mutant strains between laboratories, so as to make efficient use of them. Examples of the application of these methods to particular systems are given for defects in the visual system by Thaung *et al.* (2002) and for cardiovascular disease by Svenson *et al.* (2003). At least 1000 strains of mice derived in this manner are currently in use for functional genomics studies.

(ii) *Transgenesis and gene knockouts*

Introduction of additional genetic material to the genome can be accomplished through transgenesis. This can be achieved either by pronuclear injection, where foreign DNA is injected into a fertilized egg, by viral transduction (Whitelaw *et al.* 2004) or by transfection into ES cells. Genetic markers can be used to provide for selection of transgenic cells, before transfer to an embryo using a procedure known as *gene trapping* (Skarnes *et al.* 1995).

Alternatively, where a gene sequence is available and a targeted deletion is required in order to study the gene function, genes can be selectively removed. This is achieved by homologous recombination with a mutated version of the gene in a synthetic construct, usually in mouse ES cells, but also potentially in somatic cells for nuclear transfer. ES cells are then introduced into a conceptus and adult mice derived from them, which are bred to produce offspring homozygous for the desired deletion. Co-transfected markers such as drug resistance genes can be used to allow selection of appropriate cells, and the deletion can be verified in offspring by Southern blotting. Heterozygotes and wild-type mice of the same strain are available as controls. The phenotype of the knockout progeny reveals the gene's normal role.

However, in some cases genetic models produced in mice are not closely applicable to human pathology, as gene functions differ between the species. As a result, other species are being investigated. For instance, the human cystic fibrosis mutation does not lead to a comparably severe condition in mice, and sheep may provide a more suitable model (Harris 1997). In addition, there is a project to create a pig model of neurodegenerative disease, culminating in the birth of pigs lacking functional copies of the ataxia-telangiectasia mutant (A-TM) gene. These pigs will provide an alternative animal model in which ataxia-telangiectasia (A-T) can be studied, which the current rodent models fail to reproduce. However, the techniques for producing such targeted mutations are time consuming and unpredictable, since the inability to derive ES cells that contribute to the germ line from domestic species necessitates the use of somatic nuclear transfer.

An alternative to the mouse as an animal in which gene function can be studied is the zebrafish (*Danio rerio*). The advantages of this species are the large numbers of accessible, transparent embryos which can accelerate phenotype screening, and as a result the zebrafish is a productive model system for the genetic analysis of embryogenesis, organ development and related pathologies. Over 2000 mutations are currently available in more than 600 genes, and there is a highly developed international collaborative network in place for dissemination of these resources.

(b) *Opportunities for this century*

The deliberate production of animals with genetic disorders for research purposes clearly fails to meet goal

G2 of precision animal breeding, and random mutagenesis with subsequent identification of effects on phenotype fails to meet G1, which calls for precision in predicting breeding outcomes. For these reasons, it is appropriate that breeding under these conditions should be subject to legislation controlling the use of animals for research; in the UK, this legislation is contained within the Animals (Scientific Procedures) Act 1986. Breeding animals with defined disorders for research purposes meets the objective of the refinement, reduction and replacement (3Rs) enshrined within the Act, since more precise models of genetic disease should lead to refinement and ultimately a reduction in the numbers of experiments performed, through generation of more accurate data.

An important advance in this field will be the isolation of ES cells from livestock species. This will permit the development of animal models for human disease in a way not currently possible. However, it should be noted that although research in this area has been underway for the past 20 years, and despite the generation of chimeras, to date germ line transmission of ES cell genomic information has not been achieved in livestock. Clearly, there needs to be more research on the gene expression patterns characteristic of ES cells. While ES cells remain unavailable for livestock species and somatic cell nuclear transfer is relatively inefficient, enhancing the efficiency of targeted homologous recombination will be an important area for the future.

5. CONSERVATION BREEDING

Breeding animals for conservation differs from breeding for production purposes in three principal ways: the breeding aims are not focused on improving quantitative traits, but are based on population size and genetic variation; the numbers of individuals in endangered populations are by definition low; and stochastic environmental factors play a huge role in determining population viability. Conservation of endangered species and breeds has historically been the concern of two separate scientific communities, those involved in conservation of rare breeds of domestic animals and those working with wild animals.

(a) *Rare breeds of agricultural livestock*

About 40 species of domesticated mammals and birds are kept worldwide, although only six mammals (cattle, buffalo, sheep, goats, pigs and horses) and four birds (chickens, ducks, geese and turkeys) are widespread. Among these, approximately 7000 breeds have been produced since the beginning of domestication. In the UK, 70 breeds are recognized by the Rare Breeds Survival Trust (20 became extinct between 1900 and 1973, before the Trust was formed). The criteria for 'Rare Breed' status include a numerical basis, current trend in population size and a consideration of the period for which the population has been closed.

Efforts have been made to identify and prioritize breeds for conservation action as requiring either maintenance *in situ* or *ex situ*, or cryoconservation (a form of maintenance *ex situ*). Eding & Meuwissen (2001) explored a centrally organized approach to cryoconservation in gene banks, attempting to define objectively: (i) which breeds would or would not be stored, (ii) how many individuals to sample from each breed, and (iii) which individuals should be sampled so as to maximize the genetic variation that can be recreated from a gene bank. This method depended upon deriving relationships among breeds based upon DNA markers. Other related approaches use genetic distances (again based on markers) to define alternative measures of diversity rather than the concept of stored genetic variation that was used by Eding and Meuwissen. Weitzman (1992) provided rules for defining what may or may not be a legitimate utilitarian measure of diversity. D'Arnoldi *et al.* (1998) and Caballero & Toro (2002) provide interesting discussions on these rules. In particular, Caballero & Toro (2002) point out that diversity in a species is concerned with diversity within breeds as well as between breeds, and that this aspect is neglected in methods based on genetic distance between breeds. However, as Woolliams (2004) points out, action is required based upon the best information available at the time, using the best practice that is feasible at the time and involving stakeholders at all stages.

(b) *Endangered wild animals*

The principal threat to wild animals arises from human population growth and the aspirations of the human population for an improved quality of life. The human population, now (2006) 6.5 billion, increased exponentially from an historic base of approximately 300 million at the time of Christ to about 800 million by 1800, and increased fourfold during the twentieth century alone. It is expected to reach 9 billion by 2050. The growth in human industry (economic activity) is even more revealing than the increase in population. During the twentieth century, a fourfold increase in the population was accompanied by an 18-fold increase in the economic output. During that time, we lost about 40% of the planet's forests and 10% of the coral reefs (both major repositories of biodiversity) with additional threats to mangrove swamps, wetlands and savannah.

Extinction is of course a normal part of the life process; for example, since birds first appeared 130 million years ago, 500 000 species are said to have existed, the maximum number extant at any one time being 11 500. Today there are 9946 species of birds, about 1200 of which are threatened (listed as critical, endangered or vulnerable by the Species Survival Commission Red Data List). One hundred species have become extinct during the last 100 years. However, these statistics hide the fact that the rate of extinction, and the level of threat, is about 100-fold higher today than it has been historically, for all groups of vertebrates. For mammals, 1100 of 4763 species are threatened; for reptiles, amphibians and fishes the situation is less well known and the proportions threatened are underestimated (namely 253 out of 7970 species, 124 out of 4950 and 734 out of 25 000, respectively). The evaluation of the extinction threat to a species is an imprecise science, but has been put on a more quantitative basis by the development of criteria for endangerment by Mace & Lande (1991). The maintenance of genetic diversity is one of the goals (G3)a of precision animal breeding.

(i) Ex situ *versus* in situ *breeding*

In making breeding decisions for endangered species it is clearly advantageous to ensure the survival of animals in their home range. However, where this is not possible it is better to remove individuals to an alternative, safer, environment than to allow them to be hunted to extinction. Alternative locations include zoos or wildlife parks, and may be found in a country far from the animal's original habitat. This potentially gives rise to conflicts of interest. Removing individual animals from their home range reduces the number *in situ* thereby putting further pressure on those remaining. Where the *ex situ* site is far from the region or country of origin, there may be political pressures against removal. Local extinction following removal may result in the original habitat being irrevocably lost as a result of the lack of pressure to maintain the population locally, making reintroduction difficult. Clearly where possible it is preferable to achieve a combination of *in situ* and *ex situ* approaches.

(ii) *Conservation organizations and structures*

There are two broad categories of organization involved in the management of populations of wild animals: those based in zoos, aquaria and wildlife parks, and those overseen by the United Nations. In general, those representing the UN oversee information on a global scale, collecting and managing information with a view to developing priorities for habitat and species conservation both *in situ* and *ex situ*. Zoos, aquaria and wildlife parks principally oversee conservation breeding and management *ex situ*, but are also becoming increasingly involved in *in situ* conservation. The tools used centre on stud books, which is where the pedigree and other information is recorded, and software packages to manage the stud books.

Four United Nations bodies are involved in different aspects of conservation of biodiversity: the World Conservation Union (WCU; formally the International Union for the Conservation of Nature and Natural Resources, IUCN); the United Nations Environment Programme (UNEP); the United Nations Development Programme; and the Food and Agriculture Organization (FAO). The WCU (IUCN) established Commissions on Ecosystems Management, Education and Communication, Environmental, Economic and Social Policy, Environmental Law, the World Commission on Protected Areas and the Species Survival Commission. The last of these, the SSC, is the largest, with 120 specialist groups and task forces to identify threats to groups of taxa and recommend conservation priorities and actions. These specialist groups include the Conservation Breeding Specialist Group (CBSG) which, working through Conservation Assessment and Management Plans and Global Captive Action Plans, identifies species in need of propagation in captivity with a view to subsequent release.

The principal role of the CBSG is to facilitate decision making by arranging meetings of appropriate experts. By convening and hosting meetings of interested parties, this body develops Habitat Management Plans for individual species (population and habitat viability assessments, PHVA) or groups and geographical regions (Conservation Action Management Plans, CAMPs). PHVAs use software developed for the purpose (Vortex; http://www.cbsg.org/toolkit/vortex.scd), which applies population dynamics techniques, and takes into account demographic events at an individual animal level. The CAMPs use databases to formulate recommendations on actions required to manage populations or habitats. The level applied is wider than in the case of PHVAs, as CAMPs deal with broad taxonomic groups or geographical regions. Although their objectives appear to overlap, the structures organized by the zoo associations run in parallel with CBSG, through common membership; the same personnel are frequently involved in both sets of bodies.

Through UNEP, the World Commission on Environment and Development (the Bruntland Commission) in 1987 highlighted the importance of biodiversity and sustainability, initiating a process of international discussion culminating in the United Nations Conference on Environment and Development in Rio de Janeiro in 1992. This conference led to the Convention on Biological Diversity, which has now been ratified in 188 countries. The three objectives of the Convention are the conservation of biodiversity, the sustainable use of its components and the equitable sharing between societies of benefits arising from the exploitation of genetic resources. In 2002, the parties to the Convention adopted a strategic plan, focusing on reducing the rate of loss of biodiversity globally, regionally and locally by 2010. The seven strategies adopted as means to achieving the objectives of the Convention include: (i) 'Reducing the rate of loss of the components of biodiversity, including: (a) biomes, habitats and ecosystems, (b) species and populations, and (c) genetic diversity' and (ii) 'Promoting sustainable use of biodiversity'.

Notable advances have been made during the past 20 years in the management of endangered animals in captive populations held by zoos and wildlife parks. Stud books are held by individuals, who are most frequently animal managers and keepers in zoos. Permission to hold a stud book is agreed with the local zoo association (the European Association of Zoos and Aquaria (EAZA) in Europe or the American Zoo and Aquarium Association (AZA) in the USA), and ratified by the AZA Wildlife Conservation and Management Committee (WCMC) and the World Association of Zoos and Aquaria (WAZA). Stud books are managed using an internationally accepted software package, which includes compatible software for keeping animal records and medical records.

In North America, the AZA represents more than 200 zoos and operates for conservation breeding purposes through the WCMC. In turn the WCMC organizes Taxon Advisory Groups (TAGs, of which there are 46), which are composed of experts on particular groups of animals (taxa), recommending breeding actions to AZA institutions, evaluating the need for captive breeding and assessing the space available in zoos. Their members have expertise in taxonomy, assisted breeding, contraception and wild populations and habitats, as well as educational and training programmes. In North America TAGs develop two kinds of breeding plan, Species Survival Plans (SSPs) and Population Management Plans (PMPs).

SSPs (of which there are currently 107, representing 161 species) include genetic and demographic analyses of captive populations, and make individual breeding recommendations for both *in situ* and *ex situ* populations. PMPs (of which there are 282) fulfil the same function as SSPs, but for the *ex situ* population only. Both SSPs and PMPs work through stud books (of which there are more than 400 in North America). TAGs also develop action plans with priorities for *in situ* conservation requirements. The SSP programme started in 1981, and PMPs in 1994.

A similar structure operates outside North America. In Europe, 200 zoos in 25 countries are involved in breeding programmes. The equivalent of SSPs and PMPs are European Endangered Species Programmes (EEPs, of which there are 151) and European Stud Books (ESBs; 140), and these are run through Taxon Advisory Groups ($n=40$) reporting to the EAZA. Similar structures are in place in Africa, Australasia and Asia.

Conservation strategies for zoos worldwide are brought together by WAZA. The 2005 second edition of *Building a future for wildlife: the WAZA conservation strategy*, published by WAZA, provides a blueprint for the roles zoos and aquaria play in conservation of wildlife and their ecosystems. It represents a common philosophy for zoos and aquariums worldwide and defines the policies required to achieve their conservation goals.

(iii) *Conservation breeding and release*

There have been notable successes for conservation breeding, which have shown that with enthusiasm, commitment and an ability to collaborate, it is possible to ensure the survival of species that would otherwise have been lost. A good example is that of the Arabian oryx (*Oryx leucoryx*), a gazelle hunted to extinction in its natural range (the southern Arabian peninsula, Aden, Yemen and Oman) in 1972. A survival plan was put in place, largely initiated by the Fauna Preservation Society of the UK (now Fauna and Flora International), between 1961 and 1982 which, with 3 animals wild-caught in Aden in 1962 and 16 others from zoos taken to Pheonix, Arizona, bred a herd of 35 by 1972 and 106 by 1977. Offspring were returned to the Middle East in 1978 and to their original range in Oman in 1982. The cultural benefit of this reintroduction programme to the inhabitants of that region is hard to overestimate, and their involvement in the animals' care is evidence of the important place this oryx occupies in the culture of the desert-living nomads of that area.

Other successes of conservation breeding have been the scimitar-horned oryx, Pére David's deer, Przewalski's horse, the black-footed ferret, the Mauritius kestrel, the Puerto Rican parrot, the black and white ruffed lemur, the Egyptian tortoise, the Partula snail and the Wartbiter cricket. In each case the principles of precision animal breeding have been applied, because the animals involved were bred for a purpose and with limitation of loss of genetic variation (G3) in mind. In some cases the pedigrees of the animals used were not well known, but nonetheless, the principles were applied.

In the case of animals bred for subsequent release from captivity, there is a need for the individual animals involved to be isolated from domesticating influences (Woodworth *et al.* 2002), and to be trained in behaviours required for an unsupported existence. Will an animal raised in captivity be able to hunt, or to identify predators or poisonous plants? Examples of species in which these processes are important include most notably primates, for instance the golden lion tamarin, which has been released into its former range in Brazil.

There is also a need to carefully consider the genetic composition of the released population and its impact on the animals remaining in the captive group. Maximizing genetic diversity in the released animals may deleteriously affect that of the captive group, which should be avoided if further releases are planned, and the captive population is to be maintained. Releasing individuals over-represented by progeny benefits the captive subpopulation, because in a captive breeding programme these animals would not be bred, and their reintroduction will free spaces for further growth of the captive population. Where there is uncertainty over the survival of reintroduced animals this strategy is appropriate, because it protects the genetic diversity of the remaining captive animals. On the other hand this strategy may not maximize the survival chances of the reintroduced group, because that benefits from maximizing genetic diversity in the reintroduced animals. These trade-offs have been modelled for four captive-bred species with different breeding histories by Earnhardt (1999).

(iv) *Tissue banking and conservation by nuclear transfer*

Gamete storage techniques (sperm and oocyte freezing; germplasm preservation) have given rise to the possibility of the 'frozen zoo'. Recently this has been expanded by the opportunity to propagate nuclear DNA through somatic nuclear transfer (e.g. Gómez *et al.* 2004), offering the possibility to store tissue in the form of cell cultures, which can be frozen, for subsequent nuclear transfer to an enucleated oocyte (see §2*d*(iii)), through which cloning can benefit diversity (Woolliams & Wilmut 1999). A number of issues arise with these techniques. Firstly, it is relatively easy to freeze gametes, but more difficult to ensure they are fertile on thawing. Secondly, the numbers of endangered animals to which gametes can be transferred are limited, and this limits the information available on processes such as superovulation and induction of oestrus, which are required as part of the technique. Thirdly, somatic cells in culture may undergo chromosomal reorganizations rendering them unfit for nuclear transfer. In somatic nuclear transfer, mitochondria are transferred from the donor somatic cell to the recipient oocyte, and the offspring may be mitochondrial chimaeras; this is particularly likely if a surrogate oocyte (from a related but non-endangered species) is used as recipient. Lastly, which individual animals to preserve? There is an argument to conserve the broadest set of alleles possible from a population; appropriate animals can be identified by genotyping, by measuring a set of quantitative traits or on the basis of coefficients of relationship (Lamberson

et al. 2002). However, a random stratified sample of the population representing the gene flows in the population as identified by pedigree, performance and molecular analysis may be least at risk from deleterious genes.

The storage in frozen form of genetic material from animals is therefore much more problematic than for plants. But despite the difficulties, it does offer great opportunities, and several centres are engaged in establishing such tissue banks (including in the UK the Institute of Zoology, London and the University of Nottingham).

(c) *Opportunities for this century*

One of the key questions arising in making conservation decisions is the identity of the *ESU*, the 'population unit meriting special management' (Ryder 1986). This is defined as 'a set of populations that is morphologically and genetically distinct from other similar populations or a set of populations with a distinct evolutionary history'. This may be smaller than the species, and may not necessarily represent a reproductively isolated population: it may be based on genetic, phenotypic or behavioural criteria. The US Endangered Species Act recognizes ESUs as the unit requiring protection. The identification of the ESU for a particular species most frequently depends upon molecular genetic information, such as mitochondrial DNA and microsatellite polymorphisms. Thus, the application of molecular genetics techniques to conservation questions is of paramount importance. Examples of ESUs identified in this way are the dusky seaside sparrow and the red wolf (although in each of these cases the evidence has been challenged). An example of a species found not to represent an ESU is the Cape Verde kite (Johnson *et al.* 2005) though this study did identify two other species of kite as phylogenetically distinct, the yellow-billed kites from South Africa and Madagascar and those of northern Africa. More needs to be done to define how ESUs are determined for each species, and to apply these techniques more widely.

The developments in requirement R3 that will arise during this century will help in the recognition of selection pressures that lead to adaptation to captivity for conserved wild species. The availability of DNA information will play an increasing role in the management of diversity within an ESU (after having defined it); however it is important to recognize that the scope for managing diversity beyond best practice using pedigrees is limited (Wang & Hill 2000; Woolliams 2006). Goal G3 of precision animal breeding can be considerably advanced by more informed rural planning to avoid wild populations being fragmented into small isolated groups.

In coping with climate change and the increasing need for conservation work that will arise from it if present predictions are even close to reality, there will be a need for committed personnel, highly productive systems and structures, and well-defined objectives. Above all there will be a need for more 'spaces' for endangered animals in conservation breeding programmes.

6. COMPANION ANIMALS, PETS AND ANIMALS FOR RECREATION

Large numbers of animals are bred as pets, most frequently by professional breeders. The number bred by individuals for their own use is small by comparison. The range of species bred for this purpose is widening continually, and it is now possible to buy as pets, wallabies, miniature horses and donkeys, and a wide range of other vertebrates. Most pets, however, are dogs or cats; the size of these populations is an indication of their potential to cause mischief: there are 6.7 million dogs and 7.5 million domestic cats in the UK. There is also a feral cat population of 800 000, and each year about 135 000 dogs stray, of which about 10% are put down. There are approximately 975 000 equids (not exclusively horses). For each of these species there are examples of successes and failures in the application of the principles of precision animal breeding, sometimes with highly unfavourable genetic consequences. Online Mendelian Inheritance in Animals (OMIA; http://omia.angis.org.au/) lists more inherited disorders in dogs than any other species excluding humans and mice (479, of which only 47 have been characterized at a molecular level), with cattle second (366, 31 characterized) and cats third (273, 13 characterized). In many cases these are diseases with human homologues. Nonetheless, significant advances are now being made in the recognition of problems associated with certain breeds, and in structures which should lead to more widespread use of appropriate techniques.

(a) *Cats and dogs*

Selection in the breeding of cats and dogs is based on satisfying human aesthetic criteria rather than those ensuring fitness in a wild environment. As a result deleterious mutations have become widespread in populations, through inbreeding used to 'fix' desirable traits (Gough & Thomas 2004).

In the UK the breeding of dogs is controlled by the Breeding of Dogs Acts of 1973 and 1992, and the Breeding and Sale of Dogs (Welfare) Act 1999. This legislation licences puppy farms and ensures that the dogs are suitably accommodated, fed, exercised and protected from disease and fire. The 1999 Act provides that bitches are not mated until they are 1-year-old, that they have no more than one litter per year and that they give birth to no more than six litters in a lifetime. The Act also requires that accurate breeding records are maintained. This legislation, together with a range of other legislation relating to the welfare of farmed and non-farmed animals, is being brought together under a new bill (the Animal Welfare Bill) presently before the UK parliament. In addition to the above legislation providing for breeding of puppies, breeding of certain types of dog for fighting is prohibited under the Dangerous Dogs Act 1991, which 'prohibits persons from having in their possession or custody dogs belonging to types bred for fighting; imposes restrictions in respect of such dogs pending the coming into force of the prohibition; enables restrictions to be imposed in relation to other types of dog which present a serious danger to the public, and makes

further provision for securing that dogs are kept under proper control'.

None of this legislation covers selection in breeding, however, and as described above, many breeds now suffer from significant levels of inherited disease. The ways in which Kennel Clubs and dog shows have operated in the past have led to their rapid dissemination. Therefore, for example: (i) a number of breeds have been established with too few founders, (ii) stud books are closed for some breeds, preventing introgression of new genetic material and leading to inbreeding, (iii) selection has often been based on inappropriate type criteria, and (iv) even if the founders were sufficiently diverse genetically, clubs have frequently failed to exert adequate management of the genetic diversity generation by generation.

Kennel Clubs are generally aware of these problems. Stud books are becoming 'less closed' and phenotypic screening tests are becoming available and in use for many common inherited disorders. However, these do not identify individuals carrying recessive genes or late onset disorders which can only be diagnosed after the animal has bred. Of greater potential value are molecular markers for disease, which are currently being developed for a wide range of conditions. The development of these DNA-based tests has been facilitated by sequencing of the dog and cat genomes (Murphy *et al.* 2000; Lindblad-Toh *et al.* 2005).

These tests will identify carriers of recessive diseases such as copper toxicosis and progressive retinal atrophy, which affects several breeds, as well as mutations responsible for von Willebrand's disease in Shetland sheepdogs, Scottish terriers and Doberman Pinschers (where different mutations are responsible for the condition), phosphofructokinase deficiency syndrome in English Springer and American Cocker spaniels and pyruvate kinase deficiency in Basenjis. These tests require small blood samples and can be carried out on prepubertal animals. DNA tests for progressive retinal atrophy in Irish Setters, copper toxicosis in Bedlington terriers, and hereditary cataract and L-2-hydroxyglutaric aciduria in Staffordshire Bull terriers are already available in the UK through the Animal Health Trust and the Kennel Club. In order to speed eradication of progressive retinal atrophy in Irish Setters, there is a ban on breeding and showing carriers for the condition, which can be identified using the test. This is an example of what is achievable in this field, and it is to be hoped that other tests will be introduced with similar openness of information.

In some countries, these tests are required by law. The Netherlands and Germany have legislation banning breeding of animals with severe defects or breeding that could result in offspring with inherited disease. This is a strong implementation of goal G2. In Sweden it has been proposed to limit the number of litters per parent based on the population size for each breed, which will limit the impact of deleterious genes on the population.

There are nonetheless notable instances of excellence in breeding of dogs in the UK. An example of an organization taking a highly professional approach to breeding working companion animals is the Guide Dogs for the Blind Association. This charity breeds over 1000 guide dog puppies annually, and supports about 5000 guide dogs in use in the UK. About £10 per day is invested in the breeding, training and care of a guide dog, which is a measure of the level of investment that should be applied in breeding working dogs.

In contrast to the controls on dog breeding, the breeding of cats is uncontrolled, presumably because cats live more independent lives. Molecular approaches are less well developed than for dogs, but the mapping of the feline genome has recently been reported by Murphy *et al.* (2000), and DNA tests are being introduced (for instance the scheme for polycystic kidney disease run by the Animal Health Trust and the Langford Feline Diagnostic Service).

(b) *Horses*

Too little attention has been paid to selection in breeding sport horses. It has been suggested for some time (Cunningham 1989) that speed of racing in thoroughbreds has not improved for some years, possibly through lack of genetic variation, or from attainment of a physiological 'ceiling'. More positively, a recent study (Mota *et al.* 2002) has indicated a small genetic trend in racing time improvement, suggesting that failure to observe any phenotypic improvement may be due to only weak selection pressure since selection has been largely on prize money rather than race times. However, such trends need to be better established.

The Jockey Club rules governing thoroughbred breeding preclude artificial insemination or other assisted reproduction techniques, and this limits genetic improvement but reduces the potential to erode levels of genetic diversity. However, these rules do not apply to other breeds, and there is in particular much to be gained from a systematic approach to breeding horses and ponies for sports such as eventing and polo. This has traditionally been carried out with little or no consideration of performance testing, and as a result opportunities have been missed in international sports.

Opportunities are now emerging, however, through legislation affecting the management of horses. Following the publication by Defra of the Animal Health and Welfare Strategy for the UK in 2004, leading to the Animal Welfare bill, and as a result of concerns for the horse industry in England following the introduction of the Act prohibiting hunting with hounds, there have been two strategy documents which offer hope for the future. These reports are: *Joint Research on the Horse Industry in UK*, produced by the British Horse Industry Confederation and Defra, and the *Health and Welfare Strategy for the Horse* produced by the British Veterinary Association and Defra. As a result, there is recognition that opportunities are being lost through the fragmented nature of the UK horse industry in terms of selective breeding, and this has been addressed for eventers by the production of genetic evaluations by the British Equestrian Federation through its breeding arm, British Breeding (Kearsley *et al.* 2006). Secondly, a passport system has been introduced, which will lead to the better identification of animals, so discouraging the indiscriminate breeding of horses and ponies of low quality or genetic merit. One requirement of the passport

system is that the document should accompany the horse when it is used for breeding, thereby ensuring correct identification of the animal at that time. The legislation requires the establishment of a National Equine Database from information on passport applications, which will be a valuable tool for improvement in breeding, and, as a result, in the health and performance of competition horses and ponies in the UK. It has also been agreed that artificial insemination can be carried out safely by appropriately trained personnel, and an Exemption Order under the Veterinary Surgeons Act 1966 is currently under consideration to allow this procedure to be performed by people other than veterinary surgeons. These innovations should go some way towards redressing the historical lack of professionalism on the issue of breeding horses for sports in the UK, and help to provide structures which are taken for granted in some other European countries (e.g. Germany). They will, however, have little impact on hill breeds where animals run as herds and the assumption is that the alpha male is the sire of the foals. Although there is some indication that the herds do not mix, parentage cannot be assigned accurately on this basis.

Molecular markers have not in the past been widely used in equine breeding, but work is currently underway to map the equine genome. Supported by the Horserace Betting Levy Board and the Childwick Trust an international collaboration (including in the UK the Animal Health Trust and the Royal Veterinary College) has been established aiming to produce a low resolution genetic map for the horse. The current genetic linkage map comprises approximately 800 markers and, excitingly, the horse genome is currently being sequenced. This development should accelerate research progress in horse breeding and permit the development of genetic screening tests for inherited diseases and their use to breed healthier horses and ponies. For example, the molecular defects underlying some of the important inherited diseases in horses (hyperkalaemic periodic paralysis, severe combined immunodeficiency syndrome, lethal white foal syndrome and junctional epidermolysis bullosa) have been identified. Severe combined immunodeficiency affects 8% of Arabian horses (Ding *et al.* 2002) and a diagnostic test which will identify carrier individuals is available. An improved cytogenetic test based on chromosome painting for the X0 sex chromosome abnormality, which renders affected mares sterile, has also recently been developed, which will improve the identification of these individuals. Note added in proof: there is now a complete sequence for the horse genome.

(c) *Opportunities for this century*

Great strides have been made in the development of diagnostic tests for genetic disorders in pet and companion animals, and in the recognition of the need for progress in this area. There needs to be further work on molecular markers, and genome sequencing offers huge opportunities. Above all, realistic objectives need to be developed for the application of these tests in breeding programmes so that genetic disorders in these species become a thing of the past. Targets should be set for removing deleterious genes from susceptible breeds and for identifying individual carriers, and procedures should be implemented for recording failures of individual animals to gain pedigree status.

These targets should not be restricted to diseases that show simple Mendelian inheritance. For example, a number of bone and joint diseases pose significant health problems for horses and some have shown heritable variation in incidence: these can be addressed, for example, by expansion of bone scanning or X-raying schemes and associated record keeping, a system already in operation in Germany. Calculation of BVs for appropriate traits would aid this process. These are low-tech solutions which require structures and initiatives, not scientific advances. We now have the opportunity to reverse previous failures in addressing goal G2 in companion animals and equids, and it would be disappointing if it were not taken.

7. PRIORITIES

Priorities for the future differ between the sectors. In agricultural animals the advances in molecular biology will yield increasing benefits in genome-wide selection, but the identification of breeding objectives will be of overarching importance. To be sustainable, animal breeding needs to take account of food safety and public health, animal health and welfare, biodiversity, economic efficiency and care for the environment. Food must be nutritionally safe, free of disease and residues, and derived from animals kept in an acceptable environment. Breeding has an important role to play in limiting emissions, through improved efficiency in feed conversion and use of nitrogen and phosphate, and an improved understanding of the physiology of digestion and growth will be critical to advances in this area. All these priorities will have to be reflected in breeding objectives tailored for specific farming environments. These objectives can only be achieved by meeting the requirements for precision animal breeding, and through improved recording of information on performance and disease (R1).

There will also need to be a continuing dialogue with opinion formers in the social arena in order to ensure the techniques used and the welfare issues arising are socially acceptable. Farm animal breeding raises many questions of ethical concern, involving as it does food safety and public health, genetic selection, molecular genetics and animal welfare. This has given rise to new ways of dealing with potentially antagonistic points of view in this field, for instance the Ethical Matrix of Mepham (Mepham 2005) and the Code of Good Practice for Farm Animal Breeding and Reproduction Organizations (http://www.code-efabar.org/), which encourages discussion of the societal aspects of breeding. This is particularly important because there will be a trend towards larger companies in this field.

The question of sustainability arises most starkly in conservation breeding. In this sector, the risks and opportunities are greater than in others. The maintenance of populations of endangered species during a period of acute climate change and human population growth will call for great commitment and ingenuity, and will require the vigorous application of all the tools for precision animal breeding described here.

In other sectors advances will be made through organizational changes and with relatively low-tech innovations. Particularly in pets and companion animals, the needs are for structures and procedures to address problems currently amenable to solution through introduction of policies rather than analytical procedures. For farm animals there is a need for better recording, and the use of automated imaging and sensing techniques.

Achievement of goals will benefit from more research. We need to continue to develop the theory underpinning genome-wide selection, and the interface between population genetics and molecular genetics. However, major advances will come from the pursuit of genetical genomics and understanding the regulation of gene expression: firstly it will fill the large gap of our understanding on relating genotype to phenotype (R3), helping us to be more precise in our predictions of genetic correlations; secondly it will provide a common language for improving the understanding between geneticists and physiologists, allowing the powerful techniques of each discipline to be applied more effectively. Finally it is important for the UK and Europe to keep abreast of developments in cloning and transgenesis, and the technologies derived from them. Although cloning by somatic nuclear transfer was discovered in the UK (Campbell *et al.* 1996), it has been applied more widely elsewhere, particularly outside Europe, perhaps reflecting lack of research funding in the UK. Nevertheless it remains important to continue to develop the technology in the UK, otherwise the time may come when, as with crops, we become dependent on the rest of the world for what will one day be acceptable technologies. From research in these fields will flow developments in the other sectors dealt with here, and novel requirements, such as the need to manage environmental emissions, will also be met.

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