# New molecular challenges in animal conservation

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**Abstract**

*New molecular challenges in animal conservation.*— The contribution of genetics to wildlife conservation has been stressed often forgetting the existing theoretical and empirical limitations in the use of genetic information to solve ecological and demographic problems. The possibilities of molecular analyses are extensive and the automation of procedures is increasing the efficiency and reducing the cost of molecular technology. With large amounts of molecular data already available, the interest is switching towards the analysis of these data and the interpretation of genetic variability within and across species from a functional perspective. The understanding of the link between genetic variation and fitness or survival is essential in conservation biology and this understanding needs the combination of molecular data with non–molecular (e.g. physiological, behavioural and ecological) data. Progress in this promising field will depend on the trust and collaboration between molecular and field biologists.

Key words: Review, Molecular techniques, Animal conservation, Fitness, Genetic variation.

**Resumen**

*Nuevos retos moleculares en la conservación animal.*— La contribución de la genética a la conservación de la vida salvaje ha sido enfatizada, olvidándose a menudo que existen limitaciones teóricas y empíricas sobre el uso de la información genética para solucionar problemas ecológicos y demográficos. Los análisis moleculares ofrecen numerosas posibilidades y la automatización de los procesos está incrementando la eficiencia y reduciendo los costes de la tecnología molecular. Con grandes cantidades de datos moleculares ya disponibles, el interés se está desplazando hacia el análisis de dichos datos y la interpretación de la variabilidad genética intraespecífica e interespecífica desde una perspectiva funcional. La comprensión del vínculo entre variabilidad genética y eficacia biológica o supervivencia es esencial en la biología de la conservación, requiriendo esta comprensión la combinación de datos moleculares con datos no moleculares (por ejemplo fisiológicos, de comportamiento y ecológicos). El progreso en este campo tan prometedor debe basarse en la confianza y la colaboración entre biólogos moleculares y de campo.

Palabras clave: Revisión, Técnicas moleculares, Conservación animal, Eficacia biológica, Variación genética.

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## Rationalising the use of molecular biology

The current diversity of molecular techniques offers a wide range of possibilities to support decision makers, and genetic studies are becoming a primary argument in wildlife conservation. The importance of genetic variation in biodiversity evaluation has been recognised (EHRLICH & WILSON, 1991). Molecular biology tools have already been used to guide expensive conservation programs, including risky reintroduction projects (e.g. brown bear *Ursus arctos* [TABERLET & BOUVET, 1994]; bearded vulture *Gypaetus barbatus* [NEGRO & TORRES, 1999]). The protection of genetic diversity has been incorporated into national and international legislation.

To optimise the use of molecular biology in conservation, a wise rationalisation of the techniques and a realistic interpretation of the data produced are needed. Technological seduction and the availability of numerous informative techniques should not interfere with the recognition of the actual limitations of these techniques, both in the theoretical ground and in supporting the real problems that nature is facing (HEDRICK, 1996). For instance, it is important to recognise that molecular information might not be as critical for the immediate survival of a species as improving its habitat (CAUGHLEY, 1994) and reducing the exploitation of natural resources in this habitat (BEGON et al., 1999). Current limitations are also evident from the recognition, for instance, that no agreement has yet been reached on how to incorporate genetic diversity into land–use planning (MORITZ & FAITH, 1998).

It is also important to note that special care needs to be taken before reaching management conclusions in endangered species, where in spite of the urgency implied, erroneous recommendations could be detrimental to a species and ecosystem. Recommending the separate management of already–reduced populations could promote inbreeding. Proposing population intermixing could promote the hybridisation of specific adaptations to a particular environment (WAYNE et al., 1994).

In this work, the wide variety of molecular techniques available to support wildlife management are reviewed and relevant examples are provided in order to better understand when these techniques are used (table 1). The gap that exists between technological possibilities and their use can thus be recognized to interpret the complexity of life is noted. Finally, molecular and non–molecular biologists are appealed to collaborate in tracing the link between genes and adaptation so as to progress in many fields of life sciences including conservation biology.

## Information contained in the DNA

Variation at a given DNA region is a consequence of evolutionary forces such as mutation, selection, genetic drift or recombination that have acted over the DNA and the species (GRAUR & LI, 2000; BERTRANPETIT, 2000). Within and across populations and species the coalescense of genomic regions can be traced back and the time when genes or genome separated can be infered. Similarity relationships between DNA segments can also be evaluated to infer relationships between genes, individuals and groups of individuals. If we compare derivative characters with their geographic distribution, we can infer gene flow and colonisation events. In addition, the distribution of alleles and the structure of the genetic variation might be used to infer demographic parameters such as population size and subdivisions (LUIKART & ENGLAND, 1999).

A wide variety of polymorphic DNA regions with different mutation patterns and rates have been recognised. The choice of one or another region will depend on the objectives of our research. Most nuclear genome regions are diploid and inherited in an autosomal and codominant fashion affected by recombination. They can code for RNA or be non–coding regions. In wildlife studies, microsatellites or STRs have been widely used (QUELLER et al., 1993; LUIKART & ENGLAND, 1999). They consist of a short string of one to ten base pairs repeated in tandem and are dispersed throughout the genome. They are highly polymorphic due to the variation in the number of repeat units and most behave as neutral markers. Minisatellites are also tandemly repeated strings of longer repeat units (JEFFREYS et al., 1985). The number of repeats is inherited and variable among individuals. This variability can be detected with a probe that will attach to a single or several complementary DNA fragments among all DNA fragments distributed through an electrophoresis gel, providing a pattern of bands for comparison.

Some microsatellites and minisatellites are associated with mobile genetic elements, another DNA class that is currently gaining support for phylogenetic inference (BUCHANAN et al., 1999). These mobile or interspersed elements of different families and subfamilies occur throughout the genome. Short Interspersed Elements (SINEs) are excellent markers for molecular phylogeny since their integration at a particular position in the genome can be considered an unambiguous derived homologous character (TAKAHASHI et al., 1998). Mitochondrial DNA (mtDNA) sequences include the other major group of markers widely used in wildlife analyses (AVISE, 1994). Mitochondrial DNA is haploid, recombination free and maternally inherited. It has a low frequency of insertion, deletion and duplication events and an evolutionary rate 5–10 times higher than single copy nuclear genes (BROWN et al., 1979).

Conclusions in animal conservation should be supported by the analyses of several independent data sets (WAYNE et al., 1994). If we use different

Table 1. References with examples on the applications of molecular biology technologies to wildlife management and conservation.

*Tabla 1. Referencias con ejemplos de las aplicaciones de tecnologías de biología molecular a la gestión y conservación de la vida salvaje.*

| Technique |
|---|
| Allozymes |
| Reference: MERENLENDER et al. (1989) |
| Purpose: quantification of genetic variation and differentiation in African rhinoceroses (*Ceratotherium simum* and *Diceros bicornis)* and Asian rhinoceroses (*Rhinoceros unicornis*) |
| Results: low levels of intraspecific variation found below the levels expected in comparisons among subspecies |
| RFLPs |
| Reference: WATKINS et al. *(*1988) |
| Purpose: quantification of Major Histocompatibility Complex (MHC) polymorphism in cotton–top tamarin (*Saguinus oedipus* ) |
| Results: very low levels of polymorphism found in its MHC class I |
| DNA Fingerprinting |
| Reference: PACKER et al. (1991) |
| Purpose: study of the kinship structure in lion (*Panthera leo*) social groups |
| Results: female within the same group are closely related, whereas males can be either related or unrelated. Reproductively active males are usually unrelated to group females. Males only act as non–reproductive helpers in coalitions composed of close relatives |
| Sequencing |
| Reference: BAKER et al. (2000) |
| Purpose: determine the origins of whale products purchased from markets in Japan and the Republic of South Korea |
| Results: some protected species, such as baleen whales and sperm whales, were identified among the commercial products analysed |
| SSCP & Sequencing |
| Reference: SHAFFER et al. (2000) |
| Purpose: screening population structure and identification of management units in Yosemite toad (*Bufo canorus*) |
| Results: different genetic substructure and no shared haplotypes among animals from Yosemite and Kings Canyon National Parks. Animals from the two parks should be managed as different units |
| RAPDs, DGGE & Sequencing |
| Reference: NORMAN et al. *(*1994) |
| Purpose: analysis of population structure and identification of management units in green turtles (*Chelonia mydas*) |
| Results: Indo–Pacific rookeries include a number of genetically differentiated populations, with minimal female–mediated gene flow among them |
| RAPDs |
| Reference: NEVEU et al. (1998) |
| Purpose: comparison of the genetic diversity of wild and captive populations of mouse lemur (*Microcebus murinus*) |
| Results: captive groups have lost genetic variation in comparison with wild groups |

Table 1. (Cont.)

| Technique |
|---|
| AFLPs |
| Reference: GIANNASI et al. (2001) |
| Purpose: exploring the possibilities of AFLPs for phylogenetic reconstruction in the snake *Trimeresurus albolabris* |
| Results: *T. albolabris* is not monophyletic |
| Microsatellite analysis |
| Reference: CIOFI & BRUFORD (1999) |
| Purpose: assess the level of genetic variability and gene flow among populations of Komodo dragon (*Varanus komodoensis*) |
| Results: high levels of genetic diversity and gene flow between Rinca and Flores Islands, highest levels of genetic divergence in Komodo Island and low levels of genetic variability and gene flow in Gili Motang Island |
| Microarrays |
| Reference: TROESCH et al. (1999) |
| Purpose: genotyping and identification of *Mycobacterium* species |
| Results: the array can identify species within the genus *Mycobacterium* and detect drug–resistance |
| Minisequencing |
| Reference: MORLEY et al. (1999) |
| Purpose: assay the effectivity of fluorescent minisequencing of mtDNA for forensic use in animal, bacterial and fungal species extracts |
| Results: the technique is reliable, reproducible and suitable for forensic uses in a wide range of organisms |
| Quantitative PCR |
| Reference: FELDMAN et al. (1995) |
| Purpose: detection of malaria infection in Hawaiian birds |
| Results: avian malaria was more widespread in Hawaii than previously thought |

types of molecular data with different mutation rates we might be able to separate ancient from recent events. Another alternative is the comparison of male–inherited DNA regions (i.e. non–recombining regions of the Y–chromosome) versus female–inherited DNA regions (such as mitochondrial DNA) to understand the contribution of each sex in determining genetic diversity (MELNICK & HOELZER, 1992; PÉREZ–LEZAUN et al., 1999). This analysis can contribute to understanding how a balance is achieved between the proportion of individuals leaving the natal area and the proportion remaining philopatric to minimise inbreeding and resource competition (GOMPER et al., 1998). To identify individuals, populations or species it is often recomended to work with genetic markers that are neutral and therefore good indicators of ancestry or relationship (HEDRICK, 1996). However, there is some concern regarding how neutral characters obtained from non–coding regions reflect the diversity of functional attributes (WILLIAMS et al., 1994; LYNCH, 1996).

## Technology available

The main goal of molecular techniques is to detect the variation in DNA sequences, directly through sequencing or indirectly through other methods sensitive to sequence variations. This variation can be detected using a wide range of techniques. A first group of techniques including isozymes and restriction fragment length polymorphisms (RFLP) is based on the differential mobility of proteins and DNA fragments respectively (due to their different charge or size)

in an electrophoretic field (MÜLLER–STARK, 1998; BRETTSCHNEIDER, 1998). Hybridisation between a labeled DNA fragment or probe and a target DNA is the principle involved in many other techniques (SAMBROOK et al., 1989).

With the discovery of the polymerase chain reaction (PCR) (SAIKI et al., 1988), a new wave of molecular techniques appeared. One important advantage of the PCR is that a given DNA fragment can be isolated and copied millions of times reliably and quickly using temperature cycles and a thermally stable polymerase. This allows the use of minute amounts of DNA in molecular studies, such as those obtained from biological remnants obtained non-invasively (WOODRUFF, 1993).

Sequencing

The complete sequencing of the whole genome is the most detailed method to detect genetic variability. However, sequencing complete genomes is tedious and expensive and most studies rely on the sequencing of a minute portion of the genome and the assumption that variation within the fragment sequenced represents the variation along the whole genome. Sequencing of PCR products of up to several hundred base pairs is a widely used methodology in life sciences. During the sequencing reaction of a PCR product, a large number of fragments differing by a nucleotide in length and with the last base labelled with a specific fluorochrome depending on its identity are obtained (WEAVER & HEDRICK, 1992). When these sequencing products of different length are electrophoresed in a DNA sequencer, the ladder of fluorochrome signals obtained will indicate the nucleotide sequence of the PCR product under analysis. It is common practice to deposit the sequences obtained in public databases, facilitating both the comparison and complementation of one's own data with the data from the same or other species obtained by other researchers.

Sequencing can be combined with other methods to reduce its cost. A first group of PCR–based methods (Heteroduplex analysis, Single Strand Conformation Polymorphisms, Denaturing Gradient Gel Electrophoresis and Temperature Gradient Gel Electrophoresis) consists of screening techniques for detecting sequence variation in PCR products of identical sizes, without the need to go through sequencing. These protocols are based on the physical behaviour of DNA during electro–phoresis in acrylamide gels. The use of these methods is adequate when dealing with a large number of samples and when alleles are shared by many individuals (LESSA & APPLEBAUM, 1993).

Heteroduplex analysis

Heteroduplex analysis starts with the denaturing of the PCR product at 95°C and its subsequent renaturation before electrophoresis (LESSA & APPLEBAUM, 1993). Using this technique it is possible to distinguish between homozygous and heterozygous DNA fragments. If a sample contains two different alleles, heteroduplex molecules (hybrids of the two strands belonging to different alleles) are obtained. Since these heteroduplexes have one or more mismatches in their double strands, they migrate onto the gel more slowly than the homoduplex molecules obtained from the hybridization of strands containing the same allele.

Single–Strand Conformation Polymorphism (SSCP)

SSCP is a simple and fast method for screening DNA fragments for nucleotide sequence polymorphisms. PCR products that have been denatured by temperature and/or chemicals are loaded and run onto a non–denaturing polyacrylamide gel. The electrophoretic mobility of each single–stranded DNA fragment depends on its secondary structure, which in turn depends on its nucleotide sequence (JORDAN et al., 1998). SSCP can distinguish DNA fragments that differ only by one base-pair substitution in a fragment of up to several hundred nucleotides (ORITA et al., 1989).

Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE)

DGGE and TGGE work over double stranded DNA. In these methods, PCR products are loaded onto a polyacrylamide gel and run in a linear gradient of concentration of denaturing solvents (urea, formamide) or temperature respectively (LESSA & APPLEBAUM, 1993). The point along the gradient where the DNA fragment is partially denatured is called the melting point. This point depends on the overall base composition and the interactions across the molecule and can be modified by point mutations that will be reflected in the gel.

Randomly Amplified Polymorphic DNAs (RAPDs) and Amplified Fragment Length Polymorphisms (AFLPs)

The principle of the RAPD technique is the simultaneous amplification of DNA regions by using a single randomly chosen primer which acts as both forward and reverse (GROSBERG et. al., 1996). This primer is able to hybridise with many sites of target DNA, but amplification only occurs when the primer anneals at two sites on opposite strands separated by a reasonable distance for the PCR to work (20 to 2000 bp). These fragments are then separated in an electrophoresis gel and stained with chemicals such as ethidium bromide or silver nitrate. The gels can be scored as the presence or absence of a band of a specific molecular weight. Bands of different sizes usually represent independent loci. RAPDs are treated as neutral and anonymous markers, can be generated quickly and a large number of individuals can be processed in a

short time. However, results are difficult to repeat, a band can contain more than one amplification product that can not be distinguished and it is difficult to estimate allelic frequencies because homozygotes can not be distinguished from heterozygotes. In addition, it is sometimes difficult to know whether the variation is neutral or whether it follows Mendelian inheritance.

In AFLPs, genomic DNA is digested with restriction enzymes and the goal is to reduce the complexity of the initial mixture of fragments. To achieve this reduction a subset of fragments is biotinylated and selected by union to streptavidin-coated paramagnetic beads (since biotine binds covalently to streptavidine) (MATTHES et al., 1998). The unbound fragments are washed and discarded. A subset of the biotinylated fragments is then amplified by PCR to further reduce complexity. Finaly, PCR products are analysed by denaturing polyacrilamide gel electrophoresis and revealed by autoradiography. AFLPs are more informative and easier to reproduce than RAPDs.

## Automation required

Automation is a key issue in molecular biology and the machinery used in the automated analyses of humans and model animals is later adapted to wildlife research. Automated procedures are currently used for standard procedures such as DNA isolation or library construction and spotting but also for the fast scoring of genetic variability among individuals with technologies such as microarrays or quantitative PCR.

### Microsatellite multiplexing

Several microsatellite loci can be amplified in a single PCR reaction containing different primers (GILL et al., 1995). The primers are labeled with different fluorochromes and amplify fragments of different lengths. When the multiplex PCR reaction is run in an automated sequencer it is possible to sequentially detect the length of the different PCR products corresponding to the alleles of the different microsatellite loci.

### DNA array technology

A DNA array consists of up to thousands of DNA strings attached in order over a solid support (SOUTHERN et al., 1999). An unknown sample is passed over the array and it will hybridize upon the immobilised probes when finding a complementary sequence. The reverse is also possible when a known probe hybridises upon unknown immobilised fragments. The full microarray equipment consists of a machine to produce the array and a machine with a fluorescence laser scanner to read the signal and translate this signal to a computer. The great advantage of microarray technology is that it allows the fast detection of sequence information from a large number of loci or individuals at the same time. Paradoxically, one of the main problems encountered with microarray technology is that it generates such a large amount of information that results are often difficult to interpret. Microarrays are used, for instance, to monitor RNA expression and gene function (DE SAIZIEU et al., 1997; WODICKÁ et al., 1997; CHO et al., 1998) or to detect single nucleotide polymorphisms (SNPs) (CHAKRAVARTI, 1999). All studies published used model species and, as far as we know, no study using microarray technology has yet been performed in any species with a conservation perspective.

### Minisequencing

The technique consists of a PCR–based minisequencing reaction where the polymerase adds a single nucleotide. Primers finalise just before the polymorphic position that needs to be interrogated. The polymerase extends the first base position after the primer with labelled new nucleotides and the identity of the incorporated nucleotide can be determined with an automated sequencer. Several reactions can be performed simultaneously with primers of different sizes. It is also possible to conduct a minisequencing reaction in a DNA array (HACIA, 1999; RAITIO et al., 2001).

### Quantitative PCR

Quantitative PCR consists of a reaction that detects and quantifies nucleic acid sequences either as a final product or while the reaction is being produced. The protocol is based on the detection of fluorescence emitted by the degradation of an internal labelled oligo complementary to our sample when the PCR proceeding is being produced. The outcome is the quantification of a PCR product that can be used in gene expression studies (DE KOK et al., 2000), to evaluate viral load (LIMAYE et al., 2001), and to detect transgenes (FAIRMAN et al., 1999), duplications and deletions (AARSKOG & VEDELER, 2000; WILKE et al., 2000) and SNPs (BREEN et al., 2000). Quantitative PCR and minisequencing can be cheaper alternatives to microarrays for the study of SNPs if a moderate number of SNPs and individuals are to be analysed.

## Looking for the link between molecular data and conservation

Technological resources are available, but the connection between molecular variability and the needs of endangered species is not straightforward. Gene dynamics is complex, most phenotypic characters are multigenic, and the

genetic machinery is loaded with complicated gene interactions and epistases (HEDRICK, 1996). More than one protein can be translated from a single gene due to alternative splicing (GRAUR & LI, 2000). Genetic linkage can also mask the role of important genes. In addition, the relationship between gene and environment is often difficult to discern (FALCONER, 1989). All levels of life expression and population processes are complex and manifold and quick fixes to animal management questions based on simple molecular biology analyses should be avoided.

Genetic diversity has been linked to species richness and to better chances to cope efficiently with enviromental change (HEDRICK & MILLER, 1992; O'BRIEN et al., 1985). Consanguineous matings promote the existence of deleterious genes in homozygosis, which can be detrimental for survival and reproduction. In theory, fitness in small populations will decline due to the accumulation of detrimental mutations (LYNCH et al., 1995a, 1995b). However, the importance of genetic variability for species survival is not clearly defined. In practice, at least some populations can survive in spite of having low genetic variability (e.g cheetahs [*Acinonyx jubatus*] [O´BRIEN et al., 1987], mole–rats [*Heterocephalus glaber*] [FAULKES et al., 1990; REEVE et al., 1990] and Eurasian badgers [*Meles meles*] [DOMINGO–ROURA, 2000]). The empirical relationship between genetic distance and fitness is likely to be species–specific and is unlikely to be linear (LYNCH, 1991).

In the past, considerable effort has been devoted to describe key demographic numbers required to maintain the necessary genetic variability needed for species survival (SOULÉ, 1987). However, key numbers are unlikely to be applicable across populations or habitats. In the last decade, polemics concerning the existence of key numbers for survival have often given way to other discussions, not often based on molecular information. Within species, conservation strategies have been proposed on the basis of the existence of Evolutionary Significant Units (ESUs) which have been defined as population units that merit separate management and have high priority for conservation (RYDER, 1986). The use of ESUs in conservation has signified an upgrade from previous strategies that only gave importance to individual numbers without considering dif–ferences among individuals of the same species. However, a compromise has not yet been reached regarding the relative importance of ecological adaptation and genetic variability to determine these units (MACE et al., 1996). Furthermore, the inadequacies of the dichotomy implied in the ESU concept in a world ruled by a continuum of population differentiation have been noted (CRANDALL et al., 2000).

Across species, molecular techniques are also at the base of new strategies to support an integrated approach to conservation, focusing on the preservation of evolutionary diversity instead of focusing on species number (MAY, 1990; MACE et al., 1996) or single–species management. In this case, molecular data should play a predominant role in the selection of areas that contain evolutionarily distant lineages and areas of potential evolutionary novelty, such as multispecies contact zones (MORITZ & FAITH, 1998). The protection of these areas is likely to preserve large amounts of evolutionary heritage and will maximise the evolutionary–response potential to perturbations (PETIT et al., 1998). In fact, discussing key numbers and even single–species conservation strategies might be naif in the face of the immense complexity of nature.

## Looking for the link between gene and function

The rapid development of molecular genetics for biomedical and industrial purposes facilitates the access to molecular technology. Resolution is also increased with new techniques and a higher number of markers. The increase in the number of markers known in any species means greater probabilities to detect major loci that influence quantitative traits.

As we learn more about DNA, molecular information will be better understood when used in combination with physiological, demographic, ecological and behavioural data collected in the field (HAIG, 1998). Data can originate from any parameter that can group individuals in relation to their evolutionary origin and/or ecological needs. In animal conservation it is not enough to understand and describe molecular variation or even ecological and demographic characteristics using molecular tools. We need to find loci that have variants that are responsible for low fitness and survival. Ecologically relevant heritable traits might need to be emphasised (CRANDALL et al., 2000).

Nevertheless, fitness measurement might be difficult in endangered species. Since a selective difference smaller than the reciprocal of twice the effective population size (1/2Ne) is effectively neutral (KIMURA, 1979), small selective differences are unlikely to be of adaptive significance in most endangered species. A further complication arises from the possible differences between former and current selection and adaptation processes. The habitat currently used by a rare species can be marginal and might no longer reflect the environmental condition in which the traits evolved (JOHNSON et al., 2000). This is especially true for carnivores since human expansion has considerably altered their distribution and ecology (GRIFFITHS & THOMAS, 1993).

To unravel the link between gene and function or adaptation is not a goal exclusive to conservation genetics. For instance, to clarify the function of genes that are likely to be responsible for diseases is a major enterprise in

current biomedicine. Since the link between genotype and phenotype is still widely unknown, the potential of molecular biology in wildlife management and conservation is still at a very early stage. At this point, still far from applying the functional interpretation of genetic variation to wild species, to advance the understanding of enhanced fitness and the evolutionary paths of physiological systems, several approaches can be considered. The genetic structure of a population can be examined to identify physiological phenotypes with highest fitness. Interindividual variation can be used to identify physiological, biochemical and molecular characters that correlate with fitness and survival. Comparative studies to trace the evolution of characters with particular phylogenies can also be useful to understand the role of these characters in radiation and extinction. Other important approaches are the experimental manipulation of genes through genetic engineering, and the experimental manipulation of the environment through controlled laboratory conditions and imposed selection pressures. Even if many wildlife biologists dislike strategies such as genetic engineering, manipulative experiments and keeping animals in captivity, scientific progress will certainly be slower and may be incomplete without using these more aggressive approaches. Unfortunately, economic progress and habitat deterioration is unlikely to be slow or incomplete.

## More data, new trends

The current trend towards automation and robotisation can create important shifts in the focus of wildlife research. Highly automated laboratories are expensive but open the possibility of subcontracting services to specialised companies which can offer the same protocols than a university researcher could conduct in his or her laboratory in a cheaper, faster and often more reliable way. Some biotechnology companies are even taking a further step and sequencing interesting regions, such as regions responsible for main human diseases, in a large number of individuals, with no previous order, and selling the use of the sequences as a product. Not only this, but since many journals and common sense require sequence data be deposited in public databases, the amount of sequence data is increasing steadily. Laboratory technicians can be accurately trained to develop protocols complementary to the services offered by specialised companies. The trend is switching from young researchers who can run molecular protocols towards young researchers who can analyse molecular data generated by others. Even if this high throughput trend makes better sense when considering human molecular biology, the amount of DNA sequences from wild animals that can be found in public databases is already amazing. In addition, the dog has been suggested to be a good model for identifying the genetic control of morphologic characteristics in mammals (WAYNE & OSTRANDER, 1999). The sequencing of whole genomes for conservation purposes has not yet begun. However the proposal to start sequencing the genome of chimpanzees or macaques to understand genetic and functional differences between humans and other primates (MCCONKEY & VARKI, 2000) is likely to see the light soon.

When we leave molecular dynamics and start dealing with gene–environment interactions and adaptive characters, knowledge in other biological sciences such as ecology, zoology and behaviour becomes essential. Molecular differences have to be contrasted against non–molecular data in, for instance, geography, behaviour, morphology, and function. Applications in animal conservation only make sense when compared to field data, even if initially these data are just the species name (not always easy to determine) and the geographic origin of a sample. Accurate field data can also considerably improve the resolution of experiments. As noted by MACE et al. (1996), many studies that attempt to reconstruct familial relationships from molecular data are unable to resolve the relationship fully, even if this might have been feasible had observations been made on the breeding population to reduce the set of uncertainties for analysis.

It would be great for the progress of biological sciences and, thus, for animal conservation if samples and field data could be as easily accessible as DNA sequences to the general public. This lack of availability of samples and data creates drawbacks such as the need to spend long periods of time searching for the material required, for instance, to review the phylogeny of a doubtful taxon. This time could be devoted to more fruitful tasks if the material and accompanying data were readily available from museums and other, often public, specialized institutions. In this regard, the link between non–molecular and molecular databases is becoming an urgent need.

Successful experiments should be based in the future on a justified trust and collaboration between field and laboratory biologists. The molecular trend of research during recent years and the ease and speed with which molecular data can sometimes be published might have worked against the funding of field projects and of projects in many other areas of biology crucial for conservation biology. This trend will certainly need to be reviewed in the future when we try to translate molecular data back to nature.

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