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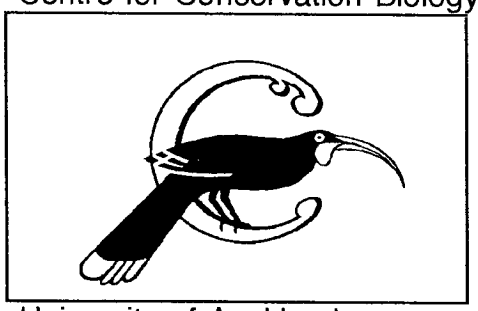

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Genetic Tools and Conservation Problems

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REVIEW

Genetic tools for conservation problems

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Preview

Exciting developments in molecular biology now provide scientists and wildlife managers with a vast array of genetic tools. These can assist us in resolving a range of contemporary conservation problems. Some of these problems have been, until recently, essentially intractable using more conventional approaches. In this article we have dealt with a number of the major recent techniques in some detail and have included an annotated bibliography of key papers which further explore aspects of these techniques. Some interesting

examples which may be a source of further reading and some works which deal with broader theoretical issues are also considered. Furthermore, molecular biology, like many other specialised fields, is littered with jargon. We have consequently provided the reader with a list of commonly used terms and their meanings. We have indicated these words with the following symbol (*).

We will attempt to provide answers to a range of common questions which scientists and conservation managers ask about conservation genetics and in so doing we hope to provide useful information about the diverse array of methodologies which are now available and to make it clear which problems we regard as relevant to each of these methods.

● What kinds of genetic techniques are currently available to study rare and threatened species?

We are not able here to consider all possible techniques. Consequently we will detail the most commonly used approaches and discuss their significant features. Table 1 summarises the major techniques currently being used and gives relevant information about the development times for these techniques, their costs, and the quality of tissue required. We will discuss each of these approaches in turn.

Isozymes

Isozyme electrophoresis uses a semisolid gel as a support medium. This enables detection of the relative positions of protein molecules after separation into discrete bands or zones. Separation of proteins occurs using a rectangular thin slab of jelly-like material, usually starch, cellulose acetate or polyacrylamide. The gel is electrified by contact with buffer tanks containing electrodes attached to a direct current power supply. The current is applied across the gel and enzymes move as a response to the potential difference which has been created.

The movement of a protein molecule in a gel is affected by (1) its electric charge, (2) the size of the protein molecule, and (3) the pore size of the gel. All proteins carry an electric charge which is determined by its amino acid composition and the pH of the buffer. In an electric field, a protein will therefore move towards the opposite pole at a rate proportional to the magnitude of its charge. Proteins with different charges will move at different rates and directions in response to an electric current. Proteins will also move at different rates depending on

their size, smaller molecules moving faster through the gel which acts as a "molecular sieve". In gels of starch and polyacrylamide, the pore size approximates that of most protein molecules. Consequently, the rate and direction of movement of proteins through these media will be determined primarily on their physical size; ie. separation is by a "sieving" process. In contrast, media such as cellulose acetate separate proteins primarily on charge.

Most proteins are not obviously visible in an electrophoresis gel, even though they have been separated as a response to the current applied. In making the enzyme visible we do not stain specifically for the enzyme itself, but for the products of enzyme activity. For example, the enzyme glutamate-oxaloacetate transaminase (GOT) breaks down alpha-ketoglutarate and L-aspartate. This enzyme converts these substrates to glutamate and oxaloacetate and is stained for by the use of the salt, fast garnet. If the enzyme (GOT) is present then the reaction will take place in the gel at the position in the gel where the enzyme is found. This consequently results in a distinctive orange colour.

Importantly, it is possible to infer the genetic basis of these enzyme patterns. This is because different alleles will often result in the production of enzymes of different size and/or charge. Therefore individuals which are homozygous for a particular allele will produce enzymes of only one mobility. Those individuals homozygous for another allele will produce enzymes of a different mobility. Heterozygotes, ie. those having one copy of each type of gene*, will produce two enzymes of different mobilities. That is, heterozygous individuals will show two bands. Such enzymes are called monomeric enzymes and are distinct from dimeric enzymes in which the heterozygotes exhibit three bands, and tetrameric enzymes in which the heterozygotes show five bands.

In a population of sexually reproducing organisms there may be a very large number of alleles at any particular locus. However, every individual will possess only two copies. For example, an individual might then be homozygous for the A allele ie. AA, or for a different allele C, ie. CC. If there are a large number of alleles in a population, there is an even larger number of possible heterozygous individuals eg. AB, AC, AD, BC etc.

Since the 1960s isozyme techniques have been used for the study of natural populations. Subsequently this technique has been applied to a range of conservation and related problems. The major uses have been: the detection of species, the estimation of the degree of structuring of populations, the investigation of phylogenetic relationships among taxa and measuring levels of genetic variation. In the detection of previously unrecognised species, there are a number of analyses which can be used. For example, a deficiency in the expected frequency of particular genotypes, or even the absence of some genotypes, may be evidence for non random mating characteristic of the existence of hidden species. Alternatively, the non random association of different genotypes (linkage disequilibrium) might also suggest the existence of a species complex. Generally the isozyme technique has been very successful at distinguishing such situations owing to the appropriate level of variation being detected at some enzyme loci. Advantages of this technique include the fact that large numbers of loci can be screened (typically in excess of 30) and therefore it is more likely that such species differences can be detected. In the key paper section we have detailed some interested articles and have drawn the readers attention to a number of the major references in this field.

In a real sense this technique has been of such widespread use in the past to solve a broad range of conservation problems because there have been, until recently, few alternative technologies. We will now discuss a range of these more recent approaches.

Mitochondrial and chloroplast RFLPs

Mitochondrial and chloroplast genomes* are small in comparison to nuclear genomes. Mitochondrial genomes range in size from 16-18kb (human, *Drosophila,* toads) to 570 kb* (maize). Chloroplast genomes, in contrast, range in size from 135-190 kb (algae) to 140 kb (maize). Approximately 2000 copies of mitochondria are found per cell (human) to 100 (yeast). In the case of chloroplasts there are approximately 100 genomes per cell (algae) to 1500 (higher plants). Owing to their small sizes these genomes can be readily separated from the high molecular weight nuclear DNA.

Furthermore, these genomes are suitable for simple restriction analysis owing to their small sizes and their high copy number per cell. Consequently,

digestion with restriction enzymes* results in the production of a number of discrete fragments.

Each restriction enzymes consistently cleaves the DNA at a specific site. In table 2. we detail restriction enzymes with a four base pair* recognition sequence which produce a number of restriction fragments in both mitochondria and chloroplasts. The larger number of chloroplast fragments is a reflection of the larger size of the chloroplast genome. In both cases six base pair* cutters result in a much reduced number of fragments. This is because there is simply a lower probability of any six base pairs being found adjacent to each other, in comparison to any four.

Since mitochondria and chloroplasts genomes are circular, a single cut site will linearise the molecule resulting in one restriction fragment equal to the size of the entire molecule. Furthermore, two restriction sites will result in two restriction fragments, the total sizes of these two molecules will correspond again to the size of the uncut genome. DNA base changes in individual molecules will result in the loss or gain of restriction sites, thus resulting in different patterns of restriction fragment length polymorphisms (RFLPs).

Fragments are then separated by size in agarose gels, small molecules moving further through the gel than larger ones. Fragments of known size are run on the same gel as a standard and allow a calibration curve to be produced which compares the relative distance each fragment migrates through the gel with the size of the fragment. Consequently unknown fragments can be accurately sized.

Chloroplasts are known to be variable in their method of inheritance. Some groups (eg. the angiosperms) have strict material inheritance, while others (eg. the conifers) have either paternal or biparental inheritance. Recombination among chloroplasts has not been reported. Mitochondrial DNA is thought to be maternally inherited without recombination in a wide variety of taxa. Interestingly, a number of different processes result in paternal mitochondria either not being packaged into the sperm head, or if they are, they are usually shed before fertilization (Lima-de-Faria, 1985). Recently, evidence has been reported of paternal mitochondrial DNA inheritance in the marine mussel *Mytilus.*

Using RFLP technology a broad array of conservation problems can be addressed. For example Baker et al. (1990) used mtDNA* RFLPs to

investigate the structure of worldwide populations of humpback whales. These authors examined a number of subpopulations which migrate to winter feeding grounds (Hawaii, Dominican Republic) from more temperate areas (Alaska, Iceland) where individuals feed during the summer months. They reported a marked clustering of mtDNA haplotypes among subpopulations as well as between populations in the Pacific and Atlantic Oceans. They argue that this structuring is a consequence of maternally directed fidelity to migratory destinations.

Bowen et al. (1992) used mtDNA restriction site variation to study worldwide populations of the green turtle (*Chelonia mydas*). This study provided evidence for relationships amongst populations and showed no evidence for matrilineal distinctiveness of a commonly recognised taxonomic form in the East Pacific.

PCR of mitochondrial DNA and sequencing

The PCR technique provides microgram quantities of DNA copies of either DNA or RNA segments which are themselves present in amounts as small as a single molecule in a nucleic acid preparation. The PCR technique is carried out *in vitro** using DNA polymerise* enzyme and oligonucleotide primers that permit the rapid and inexpensive synthesise of target DNA.

To amplify a DNA segment, two primers that are each complementary to different ends of the DNA segment are used. The PCR technique will act to amplify the sequence between the sites at which the primers anneal. After denaturation* of the target DNA the primers anneal to each strand. The sequence is then extended using DNA polymerise* enzyme when the four deoxynucleotide triphosphates* (A,T,G,C) are provided . Each primer* is extended towards the other. The resulting duplex* DNA's are then denatured and annealed again with the primers. The DNA polymerase reaction is then repeated. This cycle of steps (denaturation*, annealing* and synthesis) may be repeated as many as 60 times. At each cycle, the amount of duplex DNA segment doubles because both new and old DNA molecules anneal to the primers and are copied. In principle, and virtually in practice, 2^n copies of the duplex segment bordered by the primers are produced, where n is the number of cycles.

Using a heat stable DNA polymerase enzyme isolated from the thermophilic bacteria *Thermus aquaticus* multiple cycles can be carried out after a single addition of the enzyme. The DNA, an excess of primer molecules, the

deoxynucleotide triphosphates, and the polymerase are mixed together at the start. Cycle 1 is initiated by heating to a temperature that ensures DNA denaturation* (usually about 95°C), followed by cooling to a temperature appropriate for primer annealing* (usually about 40 - 50°C). Then the temperature is increased (usually to about 70°C) at which stage DNA synthesis occurs. The second and subsequent cycles are initiated by again heating to the denaturation* temperature. Thus, cycling can be automated by using a computer-controlled variable temperature heating block. The whole process takes only a few hours.

Besides permitting automation, use of the thermally stable polymerase has another advantage. This enzyme is most active between 70° and 75°C. At this temperature, base pairing between the primers (often about 20 base pairs long) and the DNA is more specific than at 37°C, the optimal temperature for E. coli DNA polymerase. Consequently, primers are less likely to anneal to imperfectly matched DNA segments, thereby minimising amplification* of unwanted DNA. This is important when an entire genome is present. Furthermore, correct Annealing* of primers is achieved by selecting appropriate conditions of temperature and ionic strength.

Primers can be easily designed from DNA sequence data and, in some cases from amino acid sequence data. When a number of different DNA sequences code for a particular amino acid , more than a single primer can be used. After PCR the amplified segment can be purified by gel electrophoresis or cloned* and subsequently sequenced. This can be achieved by manual sequencing or by the use of an automated sequencing machine.

PCR can also be used to amplify mitochondrial and chloroplast genomes, as well as nuclear genes. For example, Gyllensten et al. (1991) have recently used this technique to investigate the possibility of paternal inheritance of mtDNA in mice. Using PCR, paternally inherited mtDNA molecules were detected at a frequency of 10^{-4}, relative to the maternal contribution. These mice were hybrids between two inbred strains whose mtDNA could be distinguished easily. This illustrates a possible mechanism for the existence of individuals with more than one mitochondrial genotype (heteroplasmy*).

A range of other uses of PCR for conservation and ecology are now becoming available. For example, Höss et al. (1992) have described the use of PCR of mtDNA in the droppings of bears in order to identify the specific fruits which had been eaten. In addition, the technique allowed not only the identification of the

fruits, but the species of bear from which the sample was collected. In addition, Taberlet and Bouver (1992) used hair collected from wire netting on which the bears scratched themselves and used these to genetically study this endangered species.

A range of phylogenetic studies have used PCR amplifications of mtDNA and, either by direct sequencing or by cloning of the amplified fragment, have generated sequence data. The sequences from a range of taxa are characteristically then used in cladistic* or parsimony analyses in order to propose genealogical relationships. For example, Cooper et al. (1992) used sequence information from the 12S rRNA gene in the mitochondria to hypothesise phylogenetic relationships amongst the New Zealand ratites. Furthermore, John Avise (1989) from the University of Georgia has suggested that the establishment of such relationships may have important consequences in threatened species recovery programs. He asserts that a certain level of phylogenetic distinctiveness is consistently correlated with specific status. This represents a recent variation on an older theme, itself suggested by Avise, namely that specific status is related to levels of genetic distance. This idea is far from universally accepted (see Lambert and Paterson (1993) for a discussion).

In an interesting study, Allan Baker from the University of Toronto has used extensive sequence data to investigate the specific status of a number of kiwi populations and has consequently suggested that a new species is currently unrecognised (pers. comm). The implications of such work for conservation are clearly important.

Microsatellite DNA

Some molecular techniques involve the analysis of repeat regions of the nuclear genome. For example, microsatellites are regions of short simple sequence dinucleotide repeats, eg. CA_n (Weber, 1990). Microsatellites are also known as simple sequence repeats (SSRs), simple tandem repeats* (STRs), sequence tagged microsatellite sites (STMSs) or simple sequence length polymorphism (SSLPs). The latter term refers to microsatellites and minsatellites in an identical way to variable number tandem repeats* (VNTRs).

Microsatellites represent one of the most abundant families of interspersed repetitive DNA*. They are simple-sequence tandem repeat*

motifs of 1-6 DNA base pairs. For example, in an individual a CA copy may be repeated 17 times at a particular genetic locus, while on the homologous chromosome there may 10 copies. Microsatellites with 10 or more repeats have been shown to be more likely to have a greater number of alleles in the population. That is there is a relationship between increased copy number and levels of polymorphism in natural populations. Although 3-6 base pair repeats are also known, they appear to be considerably less abundant. CA sequences have been found in all eucaryotes examined to date (Tautz and Renz, 1984). The upper limit of the mutation rate for microsatellites is about 10^4 per chromosome per generation.

Microsatellites are detected using PCR with primers designed to anneal to single-copy DNA flanking the repetitive polymorphic sequence. The primers amplify the block of repetitive DNA and the different length variants are detected using polyacryamide gels and using a range of detection mechanisms. A drawback with microsatellites is that inevitably other artifactual fragments are present in the gels, although with some experience, authors have argued that most genotypes can be confidently scored.

The development of microsatellite markers requires making gene libraries of particular sizes and selecting microsatellite containing sequences. This is done by the use of repeat DNA probes and subsequent sequencing in order to develop suitable PCR primers. This is an expensive and time-consuming procedure and requires some level of technical expertise. This is a major drawback in the application of microsatellites to a wide variety of species. In addition, experience shows that these primers will not typically amplify microsatellite loci from other species (for exceptions see Morin and Woodruff (1992) where human-derived primers amplify microsatellites in chimpanzees). In birds, this has been shown to be the case and little cross-specificity has been observed (Ellegren pers communication; Ellegren, 1992). Consequently, specific primers will probably have to be developed for individual New Zealand avian species. Alternatively, Moran (1993) has recently detailed an efficient method for the design of primers for microsatellites by searching DNA sequence data bases and locating microsatellite repeat sequences together with their flanking regions. Such an approach is obviously only applicable for species where extensive sequence data are available and therefore will generally be restricted to commercially important species

such as pigs (Johansson et al 1992), chickens (Moran, 1993) and horses (Ellegren, et al 1992).

Microsatellite loci have been amplified from saliva, semen, hair follicles faecal pellets and feathers. An advantage of microsatellite techniques is that amplification* can sometimes be obtained from highly degraded tissue such as 100 year old feathers (Ellegren, 1991) and a 1,850 year old Egyptian mummy (Roewer et al., 1991). This makes microsatellite DNA techniques the most robust method with advantages over RFLPs and other PCR-based techniques such as RAPDs. In addition, DNA amounts corresponding to blood volumes as small as 0.01 ul have been successfully used in the PCR-analysis of microsatellites in birds (Ellegren, 1992).

Furthermore, microsatellites have advantages as genetic markers* in that they will sometimes reveal variation when other genetic techniques fail to do so. For example, Hughes and Queller (1993) have recently shown that microsatellites detect significant levels of variation in the social wasp (*Polistes annularis*) while isozyme techniques detected virtually no variation at all. However, microsatellites are inappropriate in other situations. For example, they are of limited use for the determination of parentage since 10 - 20 loci showing average levels of variability would be required for the unambiguous determination of maternity and paternity (Queller et al., 1993). Large numbers of useful microsatellite markers are often difficult to develop since as little as half of the isolated loci are likely to be polymorphic.

RAPDs

Randomly amplified polymorphic DNAs (RAPDs) are also refereed to as arbitrary primed polymerase chain reaction (AP PCR) or DNA amplification fingerprinting (DAF).

Oligonucleotides of approximately 10 random base pairs, when used in a PCR reaction, will often anneal to target DNA and serve as both forward and reverse primers. Three to ten DNA regions are usually amplified. These regions are typically electrophoresed in agarose and stained with ethidium bromide. A number of studies have shown moderate levels of variation in RAPD markers between individuals. These are presumably a direct reflection of sequence differences.

RAPDs initially were regarded as being a likely source of 'easy-to-produce' genetic markers applicable to a broad range of genetic problems. Because this is a PCR technique it has all the associated advantages including the need for only a small amount of potentially low-quality DNA. In addition, amplified regions can be detected by simple agarose electrophoresis*, radioactive techniques not being required.

There are a number of studies in which RADPs have been successfully employed. Welsh and McClelland (1990) used this technique to identify strains of bacteria. In addition, Crowhurst et al. (1992) were able to distinguish taxa of fungi using seven polymorphic RADP regions . They suggested that the technique is a reliable tool for typing of fungi. Hadrys et al. (1993) have recently successfully used RADPs to determine parentage in two species of dragonfly. Riedy et al. (1992) claimed that the RADP technique has deficiencies in the analysis of parentage in single offspring since they found an excess of non parental bands in offspring from know pedigrees. However, Hadrys et al. (1993) argue that parentage determination using RAPDs is suitable in species with large offspring clutches. This is only possible given that, in some species, RAPD fragments have been shown to segregate in a Mendelian fashion (see Hadry et al. (1992) for a review). However, it is important to note that in many species rapid amplification* has been shown to be inconsistent and moreover that it cannot be assumed that any amplified regions will exhibit Mendelian transmission. Consequently, it is only with known family material that this can be potentially established. Hadrys et al. (1993) claim that by the use of a "synthetic offspring" which comprises DNA from the female and the potential male parent, will compensate for amplification* artefacts that can result in non parental bands. This approach would appear to overcome the problem of non-parental bands arising as a result of the production of heteroduplex fragments, but does not overcome some of the more serious problems such as non-reproducible amplification* and, as mentioned above, non-Mendelian inheritance.

Hadrys et al. (1992) reviewed the application of RAPDs and suggested that they may be used to determine taxonomic identity, assess kinship relationships, analyse mixed genome samples, and create taxa specific probes. The main advantages, they claim, are that RAPDs are suitable for work on species with unknown genomes, that they are applicable to

problems where only limited quantities of DNA are available and lastly it represents an efficient and inexpensive technique. Hadrys et al. (1992) reviewed the limitations of the RAPD technique and discuss, in particular, the size of the primers used, the sensitivity of reaction conditions, the possibility of co-migration of fragments and non-reproducible amplification* products.

In summary, despite the initial optimism, a number of significant difficulties with RAPDs have become apparent. We suggest that only a series of careful studies will clarify the applicability of this approach.

Single locus nuclear RFLPs

As with the separation of isozymes, electrophoresis media act as molecular sieves through which smaller molecules can move more quickly than larger ones. In the case of DNA electrophoresis, agarose and polyacrylamide are typically used. Thus, over a given time, smaller fragments migrate relatively further from the origin than do larger fragments. The resolving power of the gel can be adjusted by altering the concentration of the sieving agent. Low concentrations of agarose produce a loose gel that effectively separates larger fragments, while high concentrations produce a more solid gel that resolves smaller fragments.

Typically, melted agarose is poured into a casting tray in which a plastic comb has been inserted. As it cools the agarose solidifies to a gelatinous substance consisting of a dense network of crosslinked molecules. The solidified gel is immersed in a chamber filled with buffer solution containing ions needed to conduct electricity. Removal of the comb leaves behind a series of wells into which the DNA samples are loaded. Prior to loading the digested DNA is mixed with a loading dye consisting of sucrose and one or more visible dyes. The movement of the dye allows the migration of the unseen DNA fragments to be monitored. Current is applied through electrodes at either end of the gel chamber. Following electrophoresis the DNA in the gel is chemically denatured and transferred to a solid membrane by the technique of the Southern blot*. During this process the DNA is permanently bound in a single stranded form to the membrane. Subsequently, DNA or RNA probes are then hybridised to the exposed single stranded DNA attached to the membrane. Such probes are

typically labelled with a radioactive isotope*, although a number of non radioactive techniques are becoming available. The probe* hybridises to the target DNA which has homology to it. This hybridisation is then detected by auto radiography*.

In the single locus RFLP technique the probe* is typically a short region of DNA which hybridises to DNA from alternative alleles at a single locus. Consequently, the patterns which appear are not dissimilar to those generated by the isozyme technique. A limitation of this method lies in the common need to develop probes for each particular species being studied. This has been achieved for a number of species (Quinn and White 1987a, 1987b). However the use of probes which show higher levels of polymorphism and which will cross hybridise to other species has made this a less popular method for studying genetically unknown species, although it is still useful.

Multilocus DNA fingerprinting*

This technique utilises probes specific to conserved regions within "minisatellite" DNA repeats. These regions are a category of VNTRs and are highly repeated, comprising multiple copies of a short sequence of typically less than 65 base pairs. The hypervariability is the result of a high mutation rate for the loss or gain of repeat units. Hence an individual might be heterozygous for different copy numbers ie. he or she might have received seven copies from its mother and nine from its father. Another individual might have received two from its mother and eight from its father. These regions, although being extremely variable in terms of repeat copy number, have a highly conserved 'core' region. This is a sequence of the DNA to which the probe* hybridised.

Individual minisatellite loci are considered 'hypervariable' and include the most polymorphic sequences ever detected. For example, at the most variable locus yet discovered a sample of 79 humans was found to have at least 77 different alleles. This means that the probability of any particular individual being heterozygous is 97%! Hence probes will reveal patterns of DNA fragments which are individual-specific.

Minisatellite DNA regions are not exclusive to the human genome and have been identified in a wide variety of other mammals (Jeffreys and Morton 1987)

birds (Burke and Bruford 1987), plants (Dallas 1988), fungi and protozoa. Furthermore, some of the human multilocus DNA fingerprinting probes have been shown to hybridise well to many of the different groups examined (ie they can be used as heterologous probes), in some cases detecting DNA fingerprints of similar complexity to those of humans. In particular, the original 33.15 probe isolated by Jeffreys et al. (1985a) has been an extremely useful source of genetic markers in a diverse array of organisms. DNA fingerprinting with short oligonucleotide probes which consist of simple repetitive sequences of usually no more than 5 by have been also shown to be a major source of genetic markers in a variety of plants and animals. Many of the applications of DNA fingerprinting in humans have direct parallels in animal studies.

Multilocus DNA fingerprinting has been used to establish parentage in a range of animal species (Gilbert et al. 1991; Tegelström et al. 1991). The focus of this research has however generally been directed towards the investigation of bird breeding systems. Westneat (1990) and Hunter et al. (1992) used the observed distribution of band sharing between known relatives and non-relatives to categorise individuals whose relationships to parents were in question. This general approach avoids the need for a segregation analysis and therefore is more likely to be applicable to a wider range of animal studies.

Because of the diverse array of biological systems in plants and animals and the very nature of some of the minisatellite regions detected, a number of novel applications for DNA fingerprinting have been suggested, some of which are applicable to conservation biology. For example, Kuhnlein et al. (1989) were able to calculate the genetic distances between five strains of chicken using DNA fingerprinting data and found that the genetic relationships determined from these data generally reflected the derivation of the strains. The two most genetically similar strains had been derived from a common stock, while strains of poultry derived from other stocks, and subject to different selective pressures, and different levels of inbreeding, showed greater genetic divergence. In a more extensive study Gilbert et al. (1990) examined six island populations of the California Channel Island fox (*Urocyon littoralis*) using multilocus DNA fingerprinting. These authors found relativity low levels of intra-island genetic variation, with one population monomorphic for all the restriction fragments detected. Furthermore, representatives of each population possessed a set of island-specific restriction fragments. Inter-population variation was, in contrast, high amongst the foxes examined. Both a phenetic and a phylogenetic analysis of the data grouped populations according to their

likely historical patterns of dispersal and known vicariant events. Gilbert et al. (1990) therefore suggested that DNA fingerprinting could be used successfully for phylogenetic reconstruction in small recently isolated populations.

DNA fingerprinting has been used to identify potential species, population and strain specific genetic markers in a number of organisms. For example, Meyer et al. (1991) were able to differentiate the strains of three species of filamentous fungi using DNA fingerprinting, while Nybom et al. (1989) could similarly distinguish cultivars of blackberries and raspberries.

Zeh et al. (1992) found that, despite high levels of intra-population variation in the minisatellite regions, they could clearly differentiate two populations of the pseudoscorpion *Cordylochemes scorpioides*. A population from Panama was characterised by the presence of a cluster of short restriction fragments which were virtually absent from the geographically distant French Guiana population. As a result of these differences individuals could be correctly assigned to either population. On the basis of these results and other behavioural and morphological data, Zeh et al. (1992) have suggested that *C. scorpioides* is likely to represent a complex of cryptic species.

Meng et al. (1990) examined three species of swan and suggested that the DNA fingerprint patterns detected showed a certain degree of species-specificity, and therefore could potentially reflect taxonomic affinities. However, their data were limited and far from convincing. In contrast, Weising et al. (1992) clearly demonstrated that the chickpea (*Cicer arietinum*), and a number of its relatives, can be identified as the result of species-specific banding patterns using oligonucleotide fingerprinting probes. In addition, Weising et al. (1992) found that different oligonucleotide probes revealed variation suitable for the identification of individuals and strains within the genus *Cicer*.

The above studies are a selection of some of the work being conducted on plants and animals using DNA fingerprinting. They represent examples of the diverse array of applications for which DNA fingerprinting is currently being used.

● What kinds of tissue samples are required? - Can we use non-intrusive sampling techniques eg feathers?

Table 1 details the general quality of material required for each of the techniques reviewed above. 'High quality' tissue would be that which is preserved in liquid nitrogen immediately on being taken from the animal,

or, at least, placed at -20°C (ie in an ordinary freezer) and then transferred to an ultra freezer (-80°C) as soon as possible. Material for techniques such as isozyme analyses need to be preserved in this way. Although some enzymes are more resistant to degradation than others, generally it is best to place samples immediately in liquid nitrogen (Benson and Smith, 1989). Similarly, it is possible to DNA fingerprint samples which have been preserved in buffer or kept for extended periods at room temperature, but in our experience results are much better if the tissue has been kept very cold. ' Medium' quality tissue would be, for example, that which is preserved in a Tris/EDTA buffer and then placed in an ultra freezer at a later time, or perhaps samples which have simply been kept at -20°C for extended periods. The PCR-based techniques (PARDs, mtDNA sequencing, microsatellites etc) will typically work on material that is of a very poor quality, such as museum skins (for example, see Ellegren, 1991).

Samples for liquid nitrogen storage can be placed in cryo-tubes (Nunc, agents in NZ - Gibco Life Sciences, Greiner agents in NZ - Intermed) or in eppendorf tubes. The latter have deficiencies in that they tend to explode if a hole is not made in the top prior to placing the samples in liquid nitrogen. Moreover, they are not specifically designed for use in liquid nitrogen, while the cryotubes are. The latter have an internal thread and an O ring which means that they are very resistant to cold temperatures. Despite being expensive (approximately 50 cents each) we recommend their use whenever possible.

Liquid nitrogen cylinders are often regarded as cumbersome in field situations, although it is our experience that, in many cases, the extra effort of taking a cylinder into the field pays considerable benefits later. Dry shippers are now available. These are cylinders in which the liquid nitrogen can not spill. These are more robust and less likely to break in comparison with conventional cylinders. Liquid nitrogen will be carried by commercial airlines without difficulty, simply by quoting the appropriate dangerous goods permit number UN 1977. This automatically provides the airline with details of the hazards involved. Liquid nitrogen can be obtained in New Zealand from most universities and some government organisations and from NZIG (New Zealand Industrial Gases). The latter have outlets around the country and will sell liquid nitrogen vouchers which means that you can stop at any of their nationwide centres and fill your cylinder will maximum convenience.

A range of buffers are currently in use in order to preserve material for later DNA extraction. For example, Seutin et al. (1991) provided details of an Tris/EDTA buffer which has now been widely used. Recently, Bruford et al. (1992) have reviewed the range of buffers which are now available. As we discussed above such buffers are useful in some circumstances.

● What are the relative costs of each of these techniques?

Approximate costs for each of the techniques discussed above are given in Table 1. Note that the cost estimates refer to costs after development of the technique for particular species. This is necessary because the development time for different species varies widely. Moreover, development time is, of course, difficult to accurately assess prior to the initiation of any particular project. Specialist laboratories which perform routine tests almost always do so on samples which have been collected and stored in an identical manner. Moreover, they are always derived from a single or a very limited number of species. Consequently, such laboratories can readily quote a cost per sample and guarantee results within a particular time frame. This is almost always not possible in conservation related problems.

In addition, costs depend upon many factors eg. whether labour is included, general overheads (lighting, power etc), equipment depreciation, and others. Any experimental procedures which are required to be performed on genetically unknown species, using samples collected in different ways and, for example, stored under a variety of conditions, will doubtless result in greater costs and require greater development time.

Despite all this we have endeavoured to estimate the relative costs for different techniques. 'Low' in table 1 approximates to $10-$50 per sample, 'medium' costs would be in the range of, say, $50-$200 per sample, while a 'high' is in excess of $200 per sample. An example of the highest costs would be multilocus fingerprinting for paternity purposes in humans. The commercial rate for this service is approximately $1000 for three samples. The high costs in human paternity cases are, to some degree, due to the possible need for court appearances.

● What levels of variation do each of these techniques generally detect?

The levels of variation typically recorded using these techniques are given in table 3. The general point needs to be made that the level of variation found in

any population depends, to a large degree, on the technique employed. For example, because of the processes which generate variation in minisatellite DNA, such loci tend to be highly variable. We suggest that the real question should be: what technique should we use, given that each approach will reveal a characteristic level of variation, in order to solve the specific question(s) we need to answer?

● What kinds of problems can be addressed by each technique?

Table 3 summarises the commonly-used techniques and lists some conservation problems which can be addressed using these techniques. Problems such as parentage and the identification of individuals require highly variable genetic markers. Such markers would usually be inappropriate for problems such as the detection of cryptic species. This is because, in the latter case, a marker is required which will detect genetic discontinuities at the population level. Similarly, the phylogenetic relationships between distantly related taxa require slowly evolving genetic markers. Consequently, isozymes have often been used in this regard.

● What is the relationship between genetic variability and a population's fitness?

It remains a basic assumption of almost all of evolutionary genetics, and most conservation genetics, that there is a reasonably direct relationship between the viability of a population and the level of genetic variation in that population. However, with one or two notable exceptions, correlations between these two variables in natural (as opposed to laboratory-reared) populations is not good. It is, in fact, clear that this relationship is diffuse at best. This is not surprising given that all the commonly used genetic markers are selectively neutral in that they represent genomic regions which do not undergo transcription or translation into protein products. Furthermore there is little evidence that isozymes loci are under strong selective pressure. Perhaps even more importantly, as this review of molecular techniques has demonstrated, how much genetic variation is detected in any natural population is fundamentally dependent upon the technique employed. As table 3 illustrates, different techniques will reveal different levels of variation for the same population.

● Can genetic variability be used to predict species chances of survival?

It can be seen from the above discussion that our view is that the long or medium term viability of populations is not likely to be accurately estimated by simply using of one, or even a few measures of genetic variation. Whilst the view that genetic variability is a reliable indicator of species survival is an intrinsically appealing one and is advocated by a number of contemporary authors, it has yet to be shown to be generally predictive. We believe that the array of available genetic tools have an important role to play in the resolution of a wide variety of problems of concern to conservation biologists and managers. We advocate this problem oriented approach and suggest that it offers more practical benefits for conservation scientists and managers.

Jargon

Allele	is one of several alternate forms of a gene occupying a given locus on a chromosome.
Amino Acid	A peptide; the basic building block of proteins (or polypeptides).
Amplification	refers to the production of additional copies of a DNA sequence.
Annealing	is the pairing of complementary single strands of DNA to form a double helix.
Autoradiography	detects radioactively labelled molecules by their effect in creating an image on photographic film.
Base pair (bp)	is the pairing of A with T or of C with G in a DNA double helix; other pairs can be formed in RNA under certain circumstances.
bp	is an abbreviation for base pairs; distance along DNA is measured in bp.
cpDNA	Chloroplast DNA.
Denaturation	The separation of the two strands of a DNA double helix, or the severe disruption of the structure of any complex molecule without breaking the major bonds of its chains.
D loop	is a region within mitochondrial DNA in which a short stretch of RNA is paired with one strand of DNA, displacing the original partner DNA strand in this region. The same term is used also to describe the displacement of a region

of one strand of duplex DNA by a single-stranded invader in the reaction catalyzed by RecA protein.

DNA (deoxyribonucleic acid) A double chain of linked nucleotides (having deoxyribose as their sugars); the fundamental substance of which genes are composed.

DNA fingerprint The largely individual-specific auto radiographic banding pattern produced when DNA is digested with a restriction enzyme that cuts outside a family of VNTRs, and a Southern blot of the electrophoretic gel is probed with a VNTR-specific probe.

DNA polymerase An enzyme that can synthesize new DNA strands using a DNA template; several such enzymes exist.

Electrophoresis A technique for separating the components of a mixture of molecules (proteins, DNAs, or RNAs) in an electric field within a gel.

Exon is any segment of an interrupted gene that is represented in the mature RNA product.

Gene The fundamental physical and functional unit of heredity, which carries information from one generation to the next; a segment of DNA, composed of a transcribed region and a regulatory sequence that makes possible transcription.

Gene family A set of genes in one genome all descended from the same ancestral gene.

Genetic markers Alleles used as experimental probes to keep track of an individual, a tissue, a cell, a nucleus, a chromosome, or a gene.

Genetics (1) The study of genes through their variation. (2) The study of inheritance.

Genome The entire complement of genetic material in a chromosome set.

Intron is a segment of DNA that is transcribed, but removed from within the transcript by splicing together the sequences (exons) on either side of it.

in vitro In an experimental situation outside the organism (literally, "in glass").

in vivo In a living cell or organism.

isotope One of several forms of an atom having the same atomic number but differing atomic masses.

kb	is an abbreviation for 1000 base pairs of DNA or 1000 bases of RNA.
Library	is a set of cloned fragments together representing the entire genome.
mtDNA	is mitochondrial DNA.
plasmid	Autonomously replicating extrachromosomal DNA molecule.
Primer	is a short sequence (often of RNA) that is paired with one strand of DNA and provides a free 3'- OH end at which a DNA polymerase starts synthesis of a deoxyribonucleotide chain.
probe	Defined nucleic acid segment that can be used to identify specific DNA molecules bearing the complementary sequence, usually through auto radiography.
repetitive DNA	Redundant DNA; DNA sequences that are present in many copies per chromosome set.
Restriction enzymes	recognize specific short sequences of (usually) unmethylated DNA and cleave the duplex (sometimes at target site, sometimes elsewhere, depending on type).
Restriction map	is a linear array of sites on DNA cleaved by various restriction enzymes.
Satellite DNA	consists of many tandem repeats (identical or related) of a short basic repeating unit.
SINE	Short interspersed element. A type of small repetitive DNA sequence found throughout a eukaryotic genome.
Southern blotting	describes the procedure for transferring denatured DNA from an agarose gel to a nitrocellulose filter where it can be hybridized with a complementary nucleic acid.
Splicing	describes the removal of introns and joining of exons in RNA; thus introns are spliced out, while exons are spliced together.
Tandem repeats	are multiple copies of the same sequence lying in series.
Transformation	of bacteria describes the acquisition of new genetic markers by incorporation of added DNA.
VNTR (variable number tandem repeat)	A chromosomal locus at which a particular repetitive sequence is present in different numbers in different individuals or in the two different homologs in one diploid individual.

Key Papers

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* Papers of special intetest

** Papers of outstanding interest

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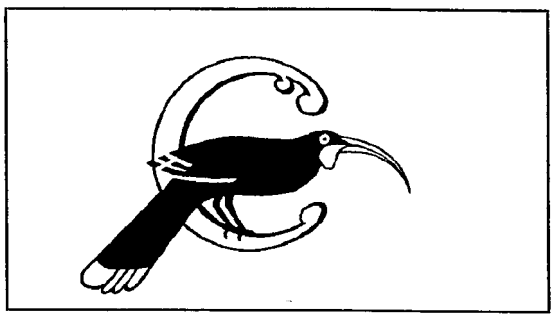
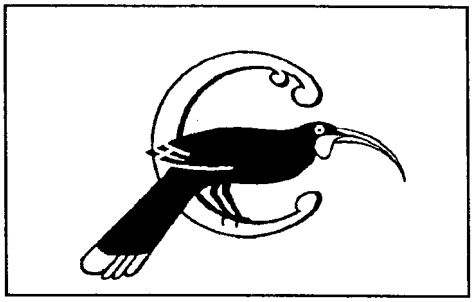
Table 1. Genetic tools and some potential uses in the study of conservation problems.



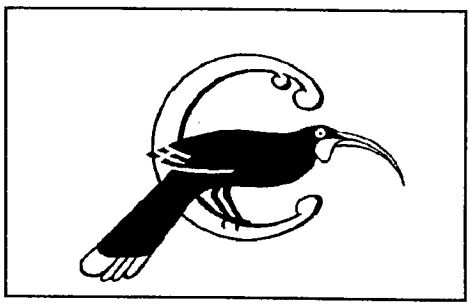
Genetic tool	Typical development time for genetically unknown species	Relative cost after development	Quality of tissue required
Isozymes	Low	Medium	High
Single Locus nuclear RFLPs	High	Medium	Medium
mtDNA and cpDNA RFLPs	Low	Low	Medium
PCR mtDNA and sequencing	Medium	Medium	Low
DNA 'Fingerprinting'	Medium	High	High
Microsatellites	High	Medium to low	Low
RAPDs	Low	Medium	Low

Table 2. Typical numbers of DNA fragments produced when human mtDNA and tobacco cpDNA is digested with restriction enzymes which have either a four or six base pair recognition sequence.



Enzyme	Recognition Site	cpDNA No. of restriction fragments	mtDNA No. of restriction fragments
4 base cutters			
Alu I	AGCT	341	64
Hae III	GGCC	196	50
Rsa I	GTAC	286	35
Taq I	TCGA	639	29
6 base cutters			
*Eco*R I	GAATTC	97	3
Hind III	AAGCTT	33	3
*Bam*H I	GGATCC	40	1
Pst I	CTGCAG	14	2

Table 3. Levels of variation detected by different genetic tools and the problems that can potentially be solved



Commonly used genetic tools	Levels of variation generally detected in natural populations	Conservation problems which can potentially be solved using these tools
Multilocus DNA finger-printing Single locus RFLPs PCR of highly variable regions e.g. mtDNA of D loop	High	Parentage Individual identification Sex assignment Feeding preferences
mtDNA RFLPs Isozymes DNA fingerprinting RAPDs Microsatellites	Medium	Detection of cryptic species Hybridisation in nature Phylogenetics of closely related taxa Population differentiation Past dispersal patterns
Isozymes PCR and sequencing of conserved regions	Low	Phylogenetics of distantly related taxa