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GENETIC DIVERSITY AND EVOLUTION

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ABSTRACT

Estimation of the amount of genetic variability in natural populations of living organisms is of such great interest that sharply divided opinion on the outcome of such estimation existed even before any reliable method of estimation was available. The biochemical technique of enzyme electrophoresis has revealed an unexpectedly high level of genetic variability in a variety of organisms. This technique suggests that organisms may be heterozygous for 6 to 15% of their loci and 15 to 50% of the loci may be polymorphic in any population. The more recent technique of DNA restriction fragment length polymorphism (RFLP) has confirmed such high levels of genetic variability. The debate now concerns, how such a high level of genetic variability is maintained. The nature of this debate is bound to have profound implications, both for evolution and for conservation. Besides, recent discoveries in eukaryote genetics are likely to upset many of our traditional views about the genetics of evolution, and hence about its relevance for conservation.

INTRODUCTION

The need for conservation

Our times have changed dramatically. Today, no one dares to ask why we should conserve life forms on earth. This may be more often due to fear of being ridiculed for asking an extremely unfashionable question rather than due to a clear understanding of the reasons behind the need for conservation. The earth has been and continues to be a storehouse of a fantastic amount of biological diversity. At least, a million and a half of living organisms have been catalogued and named. The estimated number of species of living organisms has been increasing by leaps and bounds. Not long ago, it was believed that the total number of species may be of the order of 5 to 10 million. But recent studies of beetles in the canopies of tropical rainforests suggest that there may be as many as 30 million species of insects (Erwing, 1982, 1983; Stork, 1988). Even if we accept an estimate of about 30 million for the total number of species of living organisms, it is still only a fraction of the number of species which have gone extinct during the 3.5 billion years since life originated on this planet. True, there are arguments to show that there has been a tremendous spate of extinctions in recent times and the trend is likely to continue. But one may argue that there have been such spates of mass extinctions from time to time and there is nothing unusual about the present one.

There are, however, at least two compelling arguments in favour of conservation. One is that the quality of human life and perhaps even our survival may depend on it. The second, scientifically more interesting argument, is that we may be able to prevent at least some of the extinctions. Michael Soul (1987a), has said that 'presumably, glacial and inter-glacial humans could have prevented the Pleistocene extinctions if they had our values, our knowledge of genetics, ecology, biogeography and our level of technology'. This, the author believes, is a sufficiently challenging argument to appeal even to someone who is completely unconvinced of the need for conservation of obscure forms of life.

The need for knowledge of genetics and evolution

It is an undeniable fact that most cases of conservation require money, political decisions and a certain amount of social work rather than a knowledge of genetics and evolution. Nevertheless, there are at least a few species whose conservation may be aided by an application of the principles of genetics and evolution. Species of carnivores as the cheetah (O'Brien *et al.*, 1982) primates such as the Golden Lion Tamarin (Kleimann *et al.*, 1982), birds such as the California condor (Stewart, 1978; Newton and Chancellor, 1985) and the Hawaiian goose (Stewart, 1978) can be mentioned in this context where there is sufficient interest in saving these species and there are several programmes for their conservation including attempts to breed them in captivity and release them in the wild. But, we may nevertheless be fighting a losing battle. The processes of speciation and extinction are undoubtedly governed by genetic, demographic and evolutionary principles. We may not yet have understood or discovered all of these principles but there is good reason to suspect that they exist. One may, therefore, argue that conservation biology, the science of discovering and applying the principles of genetics and evolution in conservation efforts, should be vigorously pursued. It is also true, however, that sometimes a species dwindles to such few numbers that we may be unable to apply the required biological principles. These predicaments may be expressed as the following two conundrums of conservation biology:

If all is well, you often do not need the principles of genetics and evolution.

If all is not well, you often cannot use the principles of genetics and evolution.

In spite of this, there is room to believe that there are at least a few cases which are at neither extreme, so that the principles of genetics and evolution may help.

GENETIC VARIABILITY IN NATURAL POPULATIONS

Definition of the problem

Nothing in biology makes sense except in the light of evolution, and evolution is brought about either by the force of natural selection or by random changes, the latter referred to as genetic drift. Either way, genetic variability in natural populations is the raw material for evolution. In Darwin's time, the nature of this variation was unknown. But very early in this century, it was realized that alternative forms of genes caused by mutations are the source of genetic variability. It is obvious, therefore, that any understanding of evolution requires a knowledge of the extent of genetic variability in natural populations.

The struggle to measure variation

It turns out, however, that genetic variation in natural populations is not easy to measure. Nearly all of classical genetics depended on the recognition and study of mutants that manifested themselves as morphological variants. These mutants were occasionally of spontaneous origin but were mostly induced by various mutagenic agents. Such mutants can hardly be expected to reflect the extent of natural genetic variation. Besides, these mutants only become recognizable when they lead to sufficiently strong effects. But, the stuff of evolution must be made of all those subtle variations which leave little or no trace in the external morphology of an organism. This is the epistemological paradox of Lewontin (1974) that 'what we can measure is by definition uninteresting and what we are interested in is by definition unmeasurable'.

The 'Classical' and 'Balance' hypotheses

Long before there emerged an ability to determine the extent of genetic variability in natural populations, there were two diametrically opposed schools of thought about the outcome of such measurements. The author strongly recommends a penetrating discussion on this issue by Lewontin (1974), who has argued that 'it is a common myth of science that scientists collect evidence on some issue and then by logic and intuition form what seems to them the most reasonable interpretation of the facts'. Instead, 'long before there is any direct evidence, scientific workers have brought to the issue deep-seated prejudices.....', so that 'schools of thought about unresolved problems do not derive from idiosyncratic intuitions, but from deep ideological biases, reflecting social and intellectual world views'.

According to the 'Classical' hypothesis which was largely due to Muller, two randomly chosen individuals from a population would be expected to be nearly identical at almost all their genetic loci, because so called *wild type* alleles would be expected to have gone to fixation at most loci. One of them might occasionally have a mutant allele in place of one of its *wild type* alleles. The mutant alleles are expected to be so rare that both the individuals are unlikely to have the same mutant allele. For the same reason, it would be rare to find an individual who would be homozygous for the mutant allele. Mutant alleles are expected to arise by the rare phenomenon of mutation and are expected to be eliminated from the population because most mutant alleles are deleterious. Such rare mutant alleles that are better than the *wild type* are expected to get fixed in the population, but then they would be called *wild types*. In other words, most individuals are expected to be homozygous for the *wild type* allele at most of their loci and most loci are expected to be monomorphic. The concept of genetic load that concerned Muller so much and which refers to those deleterious mutant alleles that have not yet been eliminated from the population, can be traced to this 'classical' view.

The 'Balance' hypothesis which is largely credited to Th. Dobzhansky (1955) makes a very different prediction about two randomly chosen individuals from a population. It expects that a number of different alleles exist for each locus in the population so that for most loci, the two individuals are expected to have different alleles. Even within the same individual, the two chromosomes may often have different alleles. In other words, there is no such thing as a *wild type*. Many, if not most loci, are expected to be polymorphic and any individual is expected to be heterozygous at a substantial proportion of its loci. Lewontin (1974) sees this 'Balance' hypothesis of people such as Th. Dobzhansky and E. B. Ford as reflecting the optimistic world view of natural historians that evolution is 'essentially progressive' and that it 'leads to increased harmony between living systems and the conditions of their existence'.

The electrophoretic revolution

The pioneering studies of Lewontin (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966) and of Harris (1966) which used the technique of electrophoretic detection of enzyme variants called *isoenzymes* or *isozymes* have, for the first time, made it possible to make reasonable estimates of the extent of genetic variability in natural populations. Many genes code for protein products and many proteins are enzymes that reside in the cytoplasm of the cell and contribute to one or more of the myriad steps in metabolism. Proteins are polymers of amino acids which in turn are complex organic molecules that have a net charge in solution. Although the three dimensional structure of a protein is complex and may bury many of the amino acids, the net charge of a protein molecule is usually a function of its amino acid composition and sequence. Mutations, which are changes in the nucleotide sequences of DNA, often lead to a change in the amino acid sequence of the resultant protein. This usually results in a change in the net charge of the protein molecule. Enzymes different from each other by a single mutation can often be discriminated because of their charge differences resulting in different mobilities in an electrical field. It is a well established technique today to remove enzymes from the cells, transfer them to a neutral matrix such as starch or polyacrylamide, separate them by passing electric current and visualize them by various staining procedures. As this relatively simple technique has become available, hundreds of individuals belonging to a number of species have been surveyed for variations at a variety of enzyme loci. Although all genes do not code for enzymes, and all mutations do not lead to detectable differences in the net charge of enzymes, this technique gives an approximate estimate of the genetic variability in natural populations.

Most modern studies compute two quantities namely, *polymorphism* and *heterozygosity* from such electrophoretic data. *Polymorphism* is defined as the proportion of polymorphic loci in any population of a species. A polymorphic locus is usually defined as one in which the most common allele has a frequency no greater than 0.95. *Heterozygosity* is defined as the average frequency of heterozygous individuals per locus in a population which is equivalent to the average frequency of heterozygous loci per individual. A recent survey (Ayala, 1982) indicates that 142 species have been studied and some 15 to 28 loci have been screened for each group of organisms such as fishes and amphibians. The average polymorphism ranges from 0.145 for birds to 0.529 for *Drosophila*. In general, the average polymorphism for invertebrates is 0.469, for vertebrates it is 0.247 and the plants have a value of 0.264. The average heterozygosity varies from 0.033 for self-pollinating plants to 0.151 for insects other than *Drosophila* and wasps. In general, invertebrates have a heterozygosity of 0.134 and vertebrates have a heterozygosity of 0.060 while it is 0.046 for plants. These results mean that 25 to 50% of the loci may be polymorphic and that individuals may be heterozygous at as many as 5 to 13% of their loci. This is a very high level of genetic variability indeed! Not only do these results demolish the concept of the *wild type* allele but quite clearly uphold the expectations of the 'Balance' school. In recent years, several refinements in these techniques have been made to enable the detection of more subtle changes in the charge of protein molecules. These techniques have led to a further increase, albeit a small one, in our estimates of genetic variability in natural populations.

The age of DNA

One problem with the estimates of genetic variability based on electrophoretic separation of proteins is that all DNA does not code for proteins, let alone enzymes. To obtain unbiased estimates of genetic variability, we will clearly have to look at the DNA itself. Techniques to

estimate genetic variability from restriction fragment length polymorphism (RFLP) data are being rapidly developed (Jefferys, 1979; Nei and Li, 1979). The methodology here is somewhat different. Restriction endonucleases are enzymes that cut DNA at specific sites leading to a specific number of fragments of predictable size. Changes in the sequence of DNA may abolish or create sites of recognition for these enzymes. This alters the number and size of fragments that will be obtained upon digesting DNA with restriction endonucleases. For example, Jeffery (1979) studied DNA corresponding to the globin genes of man in 60 unrelated individuals. An examination of 52 to 54 restriction sites corresponding to about 300 base pairs revealed three variants. This means that roughly one in a hundred base pairs is polymorphic. Studies at the DNA level are thus likely to further increase our estimates of the levels of genetic variability in natural populations.

What maintains genetic variability?

Far from wondering where the genetic variability is for natural selection to act upon, the electrophoretic revolution and the age of DNA have taken us to the other extreme of wondering as to why there is so much variability. There are two somewhat mutually exclusive hypotheses that attempt to explain the maintenance of this genetic variability. One is the theory of natural selection which claims that each variant is adaptive and is maintained because its bearers are fitter than those which do not have that allele. This is approximately the reasoning of the 'Balance' school which expects fitness to depend on particular combinations of alleles.

Not surprisingly, many people find it hard to believe that there is a fitness difference associated with each variant. The so called neutral theory of evolution proposes that most alleles are *neutral* and make no difference to the fitness of their bearers. Mathematical models developed by neutralists show convincingly that many neutral alleles can be maintained in natural populations due to random genetic drift and associated stochastic processes. The *selectionist - neutralist* controversy has gone on for a number of years and neither has been successfully discarded (Maynard Smith, 1989). Clearly, both processes are involved in different situations but this is not consolation for conservation biology. The implications for conservation biology of genetic variability being maintained by natural selection is quite different from the implications of variability being maintained by drift. Failure to attribute genetic variability unambiguously to one of these two forces, at least on a case by case basis, may turn out to be a significant stumbling block in applying the principles of genetics and evolution to problems of conservation.

APPLIED GENETICS AND EVOLUTIONARY BIOLOGY IN CONSERVATION – SOME GENERAL PRINCIPLES

Effective population size

It should be clear to any one that the fate of a population depends quite a bit on the number of individuals comprising the population. However, the total number of individuals in the population is not what usually matters. What really matters is the *effective population size* which may be equal to but it is often substantially lower than the actual population size. A simple example will serve to show why the effective population size can be much smaller than the actual population size. If the sex ratio in a population is biased in favour of one sex, the actual number of individuals contributing to the next generation can be substantially

reduced. An equally obvious reason for the effective population size to be less than the actual population size is that different age structures of populations result in different number of individuals in the reproducing age class. A somewhat less obvious factor that also reduces the effective population size is unequal fecundity of different individuals (Hartl, 1981).

Minimum viable populations

Many people dislike this phrase because it suggests that we can determine with certainty the minimum number of individuals that are required to prevent a population from becoming extinct. Nevertheless, this is a useful concept because it allows one to begin to model processes that determine that number. Managers of wildlife and game parks will find the phrase less offensive if they realize that the concept is more often used to help in modelling than as a firm recommendation for real life situations.

It is useful to classify factors that might lead to the extinction of a population into two categories, namely, systematic factors and chance or stochastic factors. Examples of systematic pressures on populations include deforestation, habitat destruction, hunting, etc. The principles of genetics and evolution are often of little use to mitigate such pressures on populations. It is the stochastic factors which are more troublesome and which require the principles of genetics and evolution for their understanding and possible mitigation. the stochastic factors which may drive populations to extinction, or more generally speaking, change population numbers, may be classified into four subheads (Shaffer, 1981). *Demographic stochasticity* arises from chance events that are inevitable in the survival and reproductive success of small numbers of individuals. *Environmental stochasticity* arises from chance variations in environmental factors such as the presence of predators, parasites and diseases. *Natural catastrophe* such as flood, fire and drought which may occur at unpredictable times, will also have somewhat unpredictable consequences for population sizes. Finally, *Genetic stochasticity* results from changes in gene frequencies due to genetic drift, inbreeding, etc. The purpose of the minimum viable population concept is to enable mathematical modelling of these stochastic effects on probabilities of extinction. To suit this purpose, Shaffer (1981) has proposed the following definition: 'A minimum viable population for any given species in any given habitat is the smallest isolated population having a 99% chance of remaining extant for 1,000 years, despite the foreseeable effects of demographic, environmental and genetic stochasticity and natural catas-trophes'. Using such a definition and using all available data on the grizzly bear, Shaffer (1981) has calculated that populations consisting of less than 30 to 70 individuals occupying less than 2,500 to 7,400 km^2 area, have less than 95% chance of surviving even for 100 years. The important point here is not to worry about the details of the definition of the minimum viable population because the definition can always be modified to suit particular requirements. The important point is that Shaffer and others (Gilpin and Soul, 1986; Soul, 1987b) have been developing the methodology for being able to make such calculations based on available data.

Optimum genetic relatedness to mates

It is widely recognized that *inbreeding*, defined as mating between close genetic relatives, has harmful consequences and is the main reason why small populations fail to survive for long. The idea of the deleterious effects of inbreeding is now widely recognized because it

has an apparently simple explanation. Many harmful genes are recessive and thus go undetected in the heterozygous state. But inbreeding is expected to result in homozygosity and thus lead to expression of these deleterious genes. That inbreeding is harmful, has become something of a dogma. Perhaps, this dogma is by and large justified. There is clear evidence that inbreeding leads to deleterious consequences in a variety of species (Ralls *et al.*, 1979; Ralls and Ballou, 1983). It is also well known that many species of birds and mammals avoid inbreeding (Ralls *et al.*, 1986). Apart from inbreeding leading to homozygosity of deleterious alleles, there is much evidence that heterozygosity *per se* increases the fitness of organisms (Allendorf and Leary, 1986; Ledig, 1986). The problems with such generalizations, however, is that there are significant exceptions where inbreeding and/or homozygosity are prevalent. Genetic polymorphism is virtually absent in many selfing plants, a population of elephant seal, a species of gopher, some lizards and an isolated snail population. Nevertheless these populations are doing very well (Frankel and Soul, 1981). Ralls *et al.* (1986) concluded their review of inbreeding in birds and mammals with a statement that 'it is unjustified to conclude that close inbreeding, non-incestuous inbreeding, optimal inbreeding or, indeed, optimal outbreeding prevails in natural populations of birds or mammals'. The standard explanation for such results in animals and man is that inbreeding for many generations has already eliminated most deleterious genes so that further inbreeding can not lead to homozygosity of deleterious genes. But most authors who make this suggestion are quick to add that such populations have no evolutionary potential and that they are doomed to extinction. It should be noted, however, that this last statement is a matter of belief and can not easily be scientifically tested.

The problems associated with drawing general conclusions regarding inbreeding depression are illustrated by recent studies of human populations in Southern India. The reference here is to studies being conducted at the Indian Institute of Science, Bangalore, by Appaji Rao and his colleagues. Their major findings are: i. inbreeding has been and continues to be fairly high in South Indian populations, ii. yet, there is no evidence that deleterious genes have been eliminated from these populations and iii. nevertheless, there is little evidence of inbreeding depression because there are no significant consanguinity-related differences in the survival of new-born infants (Devi and Rao, 1981; Devi *et al.*, 1982; Bittles *et al.*, 1985; Bittles *et al.*, 1987; Devi *et al.*, 1987; Rao *et al.*, 1988).

If inbreeding and the consequent homozygosity is very often detrimental to the survival of a population, does it follow then that mates should be as distantly related as possible? Apparently, the answer to this is negative. When individuals who are very unrelated to each other mate, one sees the phenomenon of outbreeding depression (Templeton, 1986). The reason usually attributed to this is that it breaks down certain combinations of genes which have evolved over long periods. One may be tempted to conclude from this that there is an optimum genetic distance for selection of potential mates. Although, this idea has some followers (Bateson, 1983) it is unlikely that there exists any such universally optimum mating distance. The consequences of inbreeding or outbreeding will almost certainly vary from species to species and even from population to population. There is likely to be no substitute therefore for basic demographic and genetic data on individual species and populations in need of conservation efforts.

The Cheetah

The cheetah has been the subject of extensive studies by O'Brien (1985, 1987b) and his colleagues using both traditional and modern techniques. Today, there are only two surviving natural populations of the cheetah, one in South Africa and the other in East Africa. Both these populations appear to be extremely genetically impoverished and reproductively impaired. Seventy percent of the sperms produced by an adult male are abnormal, a condition that would certainly lead to the verdict 'infertile' in humans and other animals. The total sperm count itself is very low; it is about 10% of what is seen in domestic cats. There is also evidence of developmental abnormalities; the cheetah skulls show significantly more asymmetry than those of other species. There is also significantly more infant mortality in captive cheetah populations compared to captive population of other cats or mammals. Two techniques show that the cheetah is genetically impoverished. The most polymorphic locus in vertebrates is the major histocompatibility complex which is responsible for rejection of tissue grafts between any two individuals except identical twins. In cheetah, however, skin grafts were accepted even from completely unrelated individuals (O'Brien *et al.*, 1985). Electrophoretic studies have led to estimates of polymorphism ranging from 0.02 to 0.04 and heterozygosity ranging from 0.0004 to 0.014. This makes the cheetah the least genetically varied species studied in the wild. From these studies O'Brien *et al.* (1987b) concluded that cheetah experienced an extreme bottleneck perhaps about 10,000 years ago and the East African population has gone through a second bottleneck within the last century.

This and other evidences can be thought of as being consistent with the idea that small populations undergo inbreeding, which in turn leads to genetic impoverishment, reproductive impairment and finally extinction. Such data are also being used to recommend outbreeding wherever possible for captive populations. For instance, it has been suggested that captive individuals derived from the South African population must be crossbred with those from the East African population. It is not clear whether such recommendations will always help. How do we know, for example, that some particular combination of genes has not been fixed in the East African population which is necessary for its survival? It should be remembered that the East African population of cheetah has survived in spite of being genetically impoverished for 10,000 years. In other words, the question is how do we avoid the pitfalls of inbreeding depression on the one hand and that of outbreeding depression on the other? There can be no general solution to this problem and only detailed studies of individual species may provide some hints.

The Asiatic lion

O'Brien and his colleagues (O'Brien *et al.*, 1987a) have also conducted a similar study with the Asiatic lion which exists in the wild as a single population of about 250 individuals in the Gir Sanctuary in India. Like the cheetah and in contrast to African lion populations, the Gir population also appears to be genetically impoverished. All 46 genetic loci studied electrophoretically were found to be monomorphic ($p = 0.0$)! Like the cheetah, the Gir lions also show a high proportion of abnormal spermatozoa. There is independent evidence that the Gir population of lions has also undergone one or more bottlenecks in the recent past.

GENETICS IS FULL OF SURPRISES

The theme of this presentation has been that the principles of genetics and evolution can, at least sometimes, be usefully applied to problems of conservation. It would be unfair to make this statement without pointing out the many pitfalls involved in applying the principles of genetics and evolution to problems of conservation. On the one hand, attempts to develop appropriate methodology to apply even the most elementary principles of genetics and evolutionary biology are still in their infancy. Most population genetic models require extremely simplifying assumptions, and are usually dubbed as 'one-locus, two-allele models'. On the other hand, our knowledge of genetics has been growing by leaps and bounds throwing up the most unexpected surprises. The following are some such important surprises that have come up as a result of recent research in genetics and molecular biology. It must be emphasized that we are very far indeed from even understanding the implications of these new findings for conservation biology. Perhaps, some of these findings will make no difference to the way in which genetic principles are applied in conservation biology. On the other hand, there are at least some which bound to have profound implications for conservation biology. But, these are decisions which can only be made after much more thought is given to this topic. Therefore, the discussion is on the most spectacular findings of modern genetics regardless of whether it appears today that they will alter the way we apply genetic principles to conservation biology. Listed below are eight statements which had more or less become dogmas in genetics and which are being questioned today (Alberts *et al.*, 1983; Lewin, 1985; Rothwell, 1988).

Double stranded DNA is the genetic material

Double stranded DNA is indeed the genetic material in most living organisms. But there are at least three kinds of exceptions to this that are known today. Many bacterial viruses such as ΦX174 have single stranded DNA as their genetic material. Many viruses have RNA (both double and single stranded) as their genetic material. Most surprisingly of all, the so called slow viruses appear to have no nucleic acids at all in them!

Information flows from DNA to RNA to protein

This is often known as the 'central dogma of molecular biology' which was unchallenged until about 1969, when Howard M. Temin discovered that information can also flow back from RNA to DNA. This pheno-menon known as reverse transcription is now well established. Once in few years, we hear of a claim that information flow from protein to nucleic acids has been demonstrated. So far these have all been false alarms but one gets the feeling that it may not be long before some form of information transfer from protein to nucleic acids is discovered.

The genetic code is universal

Genes code for the synthesis of protein by means of the genetic code which consists of triplets of nucleotides which are called 'codons' and which specify the sequence of amino acids in protein molecules. The 'meaning' of each of the possible 64 codons had been deciphered in a number of organisms during the late 1960s. Much was made of the fact that the code was universal – each three-letter codon conveyed the same message in all organisms. Among other things, the universality of the genetic code was used as evidence

to support the idea, that all life was the product of one unique event of the origin of life on earth. Today, we know that the genetic code is not universal. Many codons such as UGA, CUA, AGA, AGG and AUA appear to be used quite differently in the mitochondrial protein synthesizing machinery of some species compared to the cytoplasmic protein synthesizing machinery of all species.

The one-gene one-enzyme hypothesis

It was believed for a long time that DNA is organized into discrete non-overlapping units called genes, each of which codes for a separate protein molecule. It was soon discovered however, that some stretches of DNA did not code for either a protein or for a RNA molecule, but functioned in a structural fashion by binding to regulatory molecules. This by itself was not a big surprise. What is surprising, however, is that many genes especially in higher organisms, are not discrete non-overlapping units. Many genes have one or more stretches of 'unnecessary' sequences within them which are called 'introns'. In other words, different portions of a gene are located in several different places and have to be brought together before a protein molecule can be produced.

There is an even more profound violation of what was expected from the one-gene one-enzyme hypothesis. It was expected that the amount of DNA in an organism would be approximately equal to the amount required to code for the various proteins made by that organism. This seems to be true for a bacterium such as *Escherichia coli*. The genetic material of *E. coli* consists of a single mole-cule of DNA, 4.2×10^6 nucleotides long. This much DNA can code for 1.4×10^6 amino acids. Since an average protein in bacteria is about 350 amino acids long, the *E. coli* DNA can code for about 4,000 different protein molecules. There is evidence that *E. coli* in fact, has about 4,000 different kinds of proteins, so that it seems to have just the right amount of DNA.

If we move away from *E. coli* on either side of the complexity scale, this principle is violated. ΦX174s a very simple bacterial virus whose genome consists of a single stranded DNA molecule, 5,400 nucleotides long. This can code for 1,800 amino acids. Since the average weight of an amino acid is about 109, the ΦX174 DNA can code for a maximum of 2,00,000 daltons of protein. However, this virus is known to produce 10 proteins with a total molecular weight of about 2,50,000 daltons. In other words, this virus has less DNA than it needs. This is compensated by having overlapping genes so that some portions of the DNA are parts of two different genes and are read in different reading frames.

When we look at organisms more complex than *E. coli*, we see a different kind of violation. The genome of the fruitfully *Drosophila melanogaster* has about 1.65×10^8 nucleotide pairs per haploid genome. This can code for 55×10^6 amino acids. Even if an average protein in *Drosophila* is 700 amino acids long, this amounts to about 78,000 proteins. However, the maximum number of functional genes (proteins) in *Drosophila melanogaster* is estimated to be about 5,000. The amount of DNA present is therefore in excess of an order of magnitude more than what seems to be required. Similarly, the human genome consists of 3×10^9 nucleotide pairs per haploids genome. This much DNA can code for a billion amino acids. Even if an average protein in man is 2,000 amino acids long, the human DNA can code for 0.5 million proteins. However, the estimated number of functional genes in man is 30,000 to 40,000. Thus, 90% of the genetic material of higher organisms has no known function and is variously labelled as repetitive DNA, satellite DNA, junk DNA and more recently, selfish DNA.

DNA is a static blueprint

DNA is the blueprint which carries the master copy of nearly all the information required for the development and functioning of living organisms. It was reasonable to expect that this blueprint would not be tampered with and would remain completely unchanged throughout development and indeed throughout the life of the cell. It turns out, however, that DNA is a dynamic entity with mobile elements and jumping genes constantly moving from one location to the other and bringing about rearrangements in the blueprint itself.

The mitosis-meiosis dichotomy

It has always been believed that no genetic change takes place during mitotic cell division. It is only during meiosis that recombination and such other genetic reshuffling was expected to take place. Recent studies of plant cells in tissue culture have broken this myth. A variety of genetic changes have been detected in plant cells in tissue culture which are undergoing only mitotic cell division. Some of the genetic changes observed include aneuploidy, chromosomal rearrangements, gene introgression, sequence copy number changes, transposable element activation, new point mutations sometimes in the homozygous condition and altered expression of multi-gene families. These changes are labelled as *somaclonal variation* and have been put to much practical use (Larkin *et al.*, 1985; Larkin, 1987). Somaclonal variations in plants have been exploited to obtain increased seedling vigour in lettuce, jointless pedicels in tomato, improved rice protein content, salt tolerance and yield increase in flax, *Pseudomonas* and *Alternaria* resistance in tobacco, *Fusarium* resistance in alfalfa, etc. It is true that some of these more drastic genetic changes may be a response to the artificial conditions of tissue culture. But it is hard to believe that all of these changes are a response to tissue culture. It is reasonable to expect that at least some of these genetic changes also take place during mitotic cell divisions in plants. Genetic variability between parts of a plant such as different branches or leaves is poorly understood. But somaclonal variation seen in tissue culture should prepare us for profound genetic changes from one part of a tree to another.

Levels of natural selection

Most evolutionary arguments made are somewhat vague without precisely specifying the level at which natural selection was expected to act. The controversy over individual *versus* group selection in the last two decades has brought this problem into sharp focus and today, the individual organism rather than some higher level such as the group or the species, is thought to be the unit of natural selection. This has brought the much needed clarity in evolutionary arguments. However, it is only being realized more recently that natural selection can act on units smaller than the individual organism. To some extent, this was expected because, phenomena such as meiotic drive, gene conversion and non-reciprocal recombination have been known for some time. However, the ability of single genes or small stretches of DNA to replicate 'selfishly' at the expense and to the detriment of the organism which harbours them has come as quite a surprise. The most extreme example of such a selfish DNA is the so called paternal sex ratio factors (*psr*), which is transmitted to the zygote through the DNA in the sperms in the parasitic wasp, *Nasonia vitripennis*. Upon entering the zygote, it appears to dissociate itself from the paternal genome and selectively inactivate the rest of the paternal genome so that the zygote becomes haploid and develops into a male. This ensures that the selfish DNA can once

again reach a zygote where it can repeat the act. Populations carrying this factor thus keep on producing all-male progeny and may eventually go extinct, but laboratory stocks carrying the factor can be maintained (Warren *et al.*, 1981; Nur *et al.*, 1988).

Bottlenecks kill populations?

When a population is reduced to a very small number of individuals, it is said to have gone through a bottleneck. It is widely believed that populations that go through bottlenecks are doomed because inbreeding and other chance factors make them genetically impoverished during the bottleneck. Edwin Bryant and his colleagues (Bryant *et al.*, 1986) set out to test this prediction by rearing populations of houseflies starting from 1, 4 and 16 male-female pairs. To everyone's surprise they found that at least in some of their populations, there was more and not less variation in the bottlenecked flies in such physical characters as size and shape of their wings. Nearly all theoretical models had predicted the opposite. The simple fact is that nearly all models had assumed variance to be additive simply because it is easy to model mathematically. It appears, therefore, that non-additive variance may be more important and recent modelling efforts with non-additive variance effects come close to explaining Brayant's results. To quote Bryant, 'what happened with theoretical analysis of bottlenecks is what often happens with mathematical representations of biology ..... as often happens, you go from a simplifying process that gives you an approximate answer to thinking that you have got the answer' (Lewin, 1987). This is perhaps the single greatest danger in applying the principles of genetics and evolution in conservation biology.

IMPLICATIONS OF MODERN GENETICS FOR CONSERVATION BIOLOGY

To spell out the implications of all these surprises that modern genetics has thrown up is beyond the scope of this paper. But it is obvious that at least some of these new findings such as the dynamic state of the genome, somaclonal variation, selfish DNA and the increased phenotypic variation resulting from population bottlenecks will have profound consequences for conservation biology. Predictions of the fate of populations and estimations of minimum viable populations are bound to be very significantly affected by such processes. The task of applying the principles of genetics and evolutionary biology to problems of conservation is bound to be long and tedious with few or no shortcuts. But conservation biologists will do well to take note of the continuing advances in genetics and molecular biology.

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