

## GENERAL ARTICLES

# Genetic diversity – Conservation and assessment

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*The biodiversity that we see around us is the outcome of natural evolution which has been going on for the last 3.5 billion years. However, with growing population and rapid modernization, the biodiversity is now getting depleted at an unprecedented rate. This paper examines some of the mechanisms by which the diversity, particularly at the genetic level, is maintained under natural conditions. It discusses the phenomenon of terminal extinction as well as strategies and policies for the conservation and assessment of genetic diversity.*

THE basic characteristic of life is its unlimited diversity. No two individuals in sexually reproducing populations are the same. The underlying factor in this diversity is genetic, though other facts like age, sex, immune system, etc. do play their part in bringing about the observed differences. This biodiversity is the outcome of *natural evolution* which has been going on for the last 3.5 billion years when life first arose on this planet. Charles Darwin's theory of evolution by natural selection propounded in 1859 is predicated upon the existence of genetic diversity – a major component of biodiversity.

Since the advent of agriculture about 10,000 years ago, people have exploited the genetic variability within species to create food surpluses that made modern civilization possible. Plant and animal breeders introduced desirable genes and eliminated undesirable ones slowly, altering in the process the underlying heredity. With the emergence of new techniques of biotechnology, this process of genetic manipulation is being hastened and can be carried out with more precision than was possible with classical breeding techniques. Even the useful genes from the wild relatives of cultivated species, which do not interbreed, can be combined by protoplast fusion. All this potential depends on having access to the rich genetic diversity in nature.

However, with the growing population pressure and rapid modernization in every sphere of our activities, biodiversity is getting eroded. Land degradation, forest cutting, coastal development, environmental stress, etc. are leading to large-scale extinction of plant and animal species. The World Conservation Monitoring Centre at Cambridge, UK has estimated that 724 species of plants, fish, amphibians, reptiles, invertebrates, birds and mammals are already extinct and another 22,530 species, respectively, are threatened. Many plant and animal species, which are not in immediate danger of extinction, are suffering from declining populations. In

our country alone, which is regarded as richest in the world in flora and fauna, constituting, respectively, 7 and 64 per cent of the world's figures, the diversity is fast disappearing. Of the 45,000 plant species and 81,000 animal species, 10 and 60 per cent, respectively, are endemic and are therefore on the verge of extinction. The Earth Summit held in June 1992 at Rio de Janeiro in Brazil has therefore rightly adopted the Biodiversity Convention signed by 154 countries of the world to make sustained efforts to conserve biological diversity, to use its components on sustainable basis and to attribute value to the biological resources of the developing countries. The Government of India is also taking necessary steps for biodiversity conservation and safeguarding the cultural interests of local and regional communities.

In order to prevent depletion of biodiversity due to man-made efforts or otherwise, it is necessary to understand how the diversity of life particularly at the genetic level is maintained under natural conditions. It is equally necessary to know how the terminal extinction of species takes place under natural conditions. Based on this knowledge, one can suggest appropriate strategies and policies for the conservation of biodiversity. This paper addresses these and related issues.

## Polymorphism

In some species more than one form occurs in the same area, and these forms may be adapted to different environments in which their adaptations are maintained by natural selection. Such species are said to show *polymorphism*. It can be of two kinds, transient or balanced. In the former, a genetically controlled condition is in the process of spreading through the population and may ultimately lead to uniformity. An example of such a case is *industrial melanism* of the peppered moth. It is the name given to the phenomenon in which moths change their colour patterns from light to an all-black colouration. It is one of the best documented evolutionary changes actually witnessed, and demonstrates the

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effects of natural selection in producing adaptation conferring survival value in accordance with Darwin's theory. Tree trunks covered with lichens occurred all over Great Britain before the Industrial Revolution. The typical light-coloured peppered moth (*Biston betularia*) when resting on such a trunk or bough by day was almost invisible to birds, and thereby protected, whereas the melanic form *carbonaria* was extremely conspicuous and got rapidly eliminated. Since the Industrial Revolution, the atmosphere of many areas in Great Britain got progressively polluted by smoke. This resulted in the disappearance of visible lichens from the trunks and boughs of trees, and their darkening due to deposition of soot. The typical light-coloured peppered moth when resting on such trunks and boughs by day became now extremely conspicuous and was therefore rapidly eliminated by birds, whereas the melanic form *carbonaria* got protected in the dark background. Natural selection had thus begun to favour *carbonaria* in the new environment instead of light-coloured ones that were previously favoured.

In balanced polymorphism, on the other hand, two or more forms are maintained at reasonable frequencies in the same population. In one such condition, the heterozygote is favoured compared with either of the homozygotes, and this maintains a high degree of genetic diversity in the species. An example of this kind of polymorphism is sickle-cell in man. Among the natives of Central Africa, many have red blood corpuscles which resemble curved sickles. When such people are out of breath, their red blood corpuscles are destroyed, resulting in anemia and in serious cases, in thrombosis. The sickling is due to a single Mendelian gene, which, when inherited from one parent only, i.e. heterozygous is usually normal but when inherited from both the parents, i.e. homozygous causes death. However, the sickle-cells persist since the heterozygous carriers of the gene are more resistant to infection by the malaria parasite *Plasmodium falciparum* and are therefore favoured by natural selection. In an area of given malarial intensity, the proportion of sickle-cell people in a population reaches and maintains an equilibrium when the death rate from sickle-cell anemia exactly counterbalances the death rate from malaria.

In addition to the above examples, there are several other means of maintaining balanced or protected polymorphism such as spatial/temporal fitness variation, frequency-dependent selection, antagonistic pleiotropy, mutation-selection balance, etc. In the case of frequency-dependent selection, for instance, each allele becomes advantageous when rare but disadvantageous when frequent. This leads to stable polymorphism as shown by Wright<sup>1</sup>. In this case, the heterozygote is never fitter than both homozygotes but has a fitness which is always exactly halfway between those of the two homozygotes.

The subject of balanced polymorphism, which is in fact just one facet of the maintenance of genetic variability in natural populations, has been extensively studied since the 1940s. Experimental investigations on natural populations of several organisms have indicated abundant genetic variation for Mendelian traits like allozymes as well as for most of the quantitative traits. To explain the nature of this genetic variability, mathematical analyses based on different models have been attempted by different workers since the 1920s. Narain<sup>2</sup> gives a detailed review.

One of the mechanisms proposed is the balancing between forces of stabilizing selection and mutation. The former acts against deviants from an optimal value, and so eliminates genetic variability. The latter provides new deviants restoring this variability, thus leading to an equilibrium. For infinitely large populations (i.e. with no random drift), the models differ in the number of alleles at a locus, the mutation scheme and the nature of time parameter. Di-allelic multi-locus models investigated by Latter<sup>3,4</sup>, Bulmer<sup>5,6</sup> and Barton<sup>7</sup> led to the conclusion that the equilibrium genetic variance is independent of the allelic effect but depends on the mutation rate, intensity of selection and the number of loci. The infinitely many alleles model introduced by Kimura<sup>8</sup>, on the other hand, considered a continuous time parameter where alleles are distinguished according to the distribution of their additive effects and their frequencies do not enter into the analysis. At equilibrium the distribution of effects is Gaussian with genetic variance depending upon the mutation rate, the variance of the mutational change, the intensity of selection and the number of loci. Turelli<sup>9</sup> introduced an alternative *house of cards* approximation for such a problem. Based on the premise that the variance of the mutational effects at a locus is much larger than the genetic variance at that locus, such an approximation led to the prediction of equilibrium genetic variance which is identical to that of the di-allelic model, thus indicating that the equilibrium genetic variance is independent of the number of alleles considered at a locus. A discretized version of the continuum-of-alleles model was given by Narain and Chakraborty<sup>10</sup>, Slatkin<sup>11</sup> and Narain<sup>12,13</sup>. This model was essentially a step-wise mutation model with discrete allelic effects and stabilizing selection. Initially the number of alleles considered was taken as infinitely large, but in Narain<sup>14</sup>, a finite number of alleles was also considered. The results obtained were found to be more general and encompassed on the one hand, Turelli's<sup>9</sup> findings based on the *house-of-cards* approximation for strong selection, and on the other, the results of the normal approximation for weak selection. It seems the number of alleles considered at each locus could be a crucial factor in mutation-selection balance provided forces are sufficiently large that no more than two alleles can segregate at the locus.

A related aspect of polymorphism, wherein polymorphic characters affect survival and fertility of the organism, was first pointed out by Ford<sup>15</sup> but was not taken seriously. Subsequently, Clark *et al.*<sup>16</sup> reported association between some of the blood groups and common diseases like cancer of the stomach, gastric ulcer and diabetes mellitus. With the advent of biochemical techniques and molecular biotechnology, this subject has advanced considerably as we will discuss briefly next.

### Assessing genetic diversity in natural populations

The technique of *gel electrophoresis* enables us to measure genetic variation in a natural population by sampling about 50 or more individuals and identifying the genotypes of each individual in respect of about 20 or more genes coding for an equivalent number of proteins or enzymes. The frequency of heterozygotes for each gene is first determined and then the average heterozygosity for all the genes in the sample is calculated. Several scientists working in different laboratories have measured genetic variation in several organisms using this technique. Reliable estimates of average heterozygosity are now available for more than 100 species of plants and animals. A great deal of genetic variation is found in most natural populations of sexually reproducing organisms. On the average, invertebrate organisms are heterozygous at about 13 per cent of their loci. On the other hand, vertebrates are less polymorphic, being heterozygous for about 6 per cent of the loci. On an average, human beings are heterozygous for about 6.7 per cent of the loci. Plants, on the contrary, show a rather high degree of heterozygosity.

In addition to the enzyme or protein polymorphism, recent advances in biotechnology have enabled use of new types of polymorphisms discovered at the DNA level. Bacterial restriction endonucleases, which cleave DNA at sequence-specific sites, break down a very long DNA molecule into small fragments. If the variation in the DNA sequence at a particular locus is such that one of the variants is cleaved by a restriction enzyme and the other is not, then the variant that is not cleaved at that locus will be associated with a longer fragment of DNA. This kind of sequence variation is known as *Restriction Fragment Length Polymorphism* (RFLP). Such markers are found in the coding as well as non-coding regions of the DNA. They are usually biallelic and co-dominant so that they allow all the three genotypes to be discriminated.

Another powerful technique for generating polymorphism is that of the *Polymerase Chain Reaction* (PCR) which requires much less DNA to produce a detectable band on the film. It amplifies a segment of DNA flanked by two specific sequences. It has proved useful

in generating markers known as *Random Amplified Polymorphic DNA* (RAPD). Such markers are essentially two-allele loci with presence of the two recognition sequences dominant to absence. At certain loci in the genome, the variation between individuals occurs in the form of a variable number of repeats of a particular sequence of base pairs. For minisatellites or *Variable Number of Tandem Repeats* (VNTR), the repeat unit is of the order of 10 bp and the number of units can be in thousands. Micro-satellites or *Short Tandem Repeats* (STR) or *Simple Sequence Repeats* (SSR) have repeat units of only 2 or 3 bp. Each of the possible number of repeats in VNTR or STR represents a different allele. At each locus, therefore, we can have a very large number of alleles. The main method of detecting STR is based on PCR.

The RFLP, RAPD and hypervariable (VNTR and STR) markers have been found to be extremely useful in linkage analysis for mapping of the mutations responsible for hundreds of Mendelian diseases. The RFLP and RAPD markers have also been found useful in the identification of *Quantitative Trait Loci* (QTLs) by means of correlation between the trait and the marker. In plants and animals, the RFLP maps can be used to select the desired gene combinations in an indirect manner from a breeding population, usually known as *Marker-Assisted Selection* (MAS). It has also been useful in selection of genotypes in early generations from a segregating population and is being used in breeding improved varieties of a number of crop plants across the world. The first most notable achievement in this regard has been in tomatoes<sup>17</sup> wherein QTLs governing fruit mass and fruit pH have been identified and selected for high solid content.

The high level of genetic variation discovered in natural populations indicates that these populations have plenty of scope for evolution to occur. Whenever a new environmental challenge appears – whether due to climate change or man-made pollution or due to any other cause – the populations are usually able to adapt to it. The evolution of resistance to insecticide in insect species (recently reported in more than 200 species) or heavy metal tolerance in plants or antibiotic resistance in bacteria is a proof of this adaptation. Further in out-crossing organisms, the high degree of genetic variability provides the genetic basis of individuality. In case of human beings, for instance, if the genome is taken to consist of 10,000 allelic pairs coding for proteins, the average heterozygosity of 6.7 per cent amounts to producing an immensely large number of 10,200 gametes.

### Extinction of species

There are two types of extinction of species generally thought to be very rare. Firstly, a plant or animal spe-

cies could be transformed over a number of generations into a physically distinct descendant by natural selection. The species gradually adapts to its changing environment. Secondly, there could be terminal extinction as the outright elimination of a species. There have been five such large-scale mass extinctions in the last 500 million years. There is no unanimity in the cause of such mass extinctions. Asteroid impacts is one possibility which in fact coincided with the disappearance of dinosaurs 65 million years ago. In none of these large-scale extinctions, did humans play any role. The Neolithic hunters came on the scene only 10,000 years ago which is not a long time in the history of life. Against this background, the present-day endangered species and depletion of biological diversity is quite a serious matter. With the loss of genetic variability within a species, the pool of genetic resources for further evolution is gradually drained. The loss of every gene, species or ecosystem limits our option for the future.

### **Erosion of genetic diversity due to size bottleneck**

It is well known that inbreeding in small captive populations has resulted in deleterious effects and loss of fitness of the population. In natural populations too, severe reductions in population size, known as *genetic bottleneck*, have led to loss of genetic diversity, thereby increasing the susceptibility to infectious diseases and the consequent increased chances of extinction. Genetic models predict that the proportion of initial heterozygosity retained per generation is  $[1 - (1/2N_e)]$  where  $N_e$  is the effective population size, usually less than  $N$ , the actual population size. Thus a population of  $N_e = 10$  individuals loses 5% of its heterozygosity per generation. This indicates that severe bottlenecks degrade heterozygosity<sup>18</sup>.

### **Biotechnology and genetic diversity**

We have already discussed some aspects of biotechnology in terms of RFLP, RAPD, etc. The unique potential of biotechnology arises from the fact that underlying nature's diversity, there is a unity in the way genetic material encodes and expresses the instructions for growth and development. The genes which constitute the chromosomes of diverse organisms like wheat and humans are made of the same building blocks and operate according to similar rules. There is an inherent unity in the chemistry of living organisms. The most widespread application of biotechnology is that of the gene splicing technique which enables us to insert genes from one species into another species which traditionally was not possible to combine.

In plant breeding, scientists are attempting to isolate the genes carrying apomixis (asexual reproduction through seed), clone them and introduce them into crops which have no apomictic relatives. As is well known, apomixis is a great tool in the hands of a plant breeder. It is used to create hybrids which retain their vigour for generations as well as to lock traits such as high yields, disease and insect resistance and other improvements in important food and fodder crops. Apomixis fixes at once any desirable new combinations. It has been identified in more than 35 families of plants including more than 300 species. It is common in citrus and has been identified in the wild relatives of cultivated crops including corn, pearl millet, and wheat as well as strawberry, mango and beet. It is usually expressed in *polyploids* (plants having more than two chromosomes of each kind) but attempts are now being made to introduce it in maize which is a *diploid*.

Although apomixis has great potential in broadening the accessibility of hybrids, it is a threat to biodiversity. As it involves cloning of plants through seeds, all offspring are identical to the mother plant. This means narrowing the genetic base and susceptibility to disease. Well known examples are *potato blight* and southern *corn leaf blight*. As such it is essential that a balance is kept between sexual and apomictic plants in varieties released commercially. In fact, in most successful, naturally occurring apomictic species there is an equilibrium between sexual reproduction and apomixis. We need therefore to determine what percentage of apomixis can be released without endangering genetic diversity.

Biotechnology applied to animal husbandry involves design and production of new vaccines, actual transfer of genes between species and engineering the normal reproductive process. The last one, built on the foundations of artificial insemination, refers to *embryo collection and transfer* and converts prize cows into superovulators, generating 40 or more calves a year without actually having to give birth. Although this technology reduces the time in which a herd can be improved with the genetic traits of superior parents, it also hastens the elimination of unidentified potentially useful genetic traits from a herd.

### **Strategy and policies for the conservation of biodiversity**

There is a widespread belief both in India and abroad that the conservation of biodiversity can be achieved by having concern for the protection of nature and launching a movement to this end. Several NGOs have sprung up to raise slogans for the conservation of biodiversity. While this is necessary to draw the attention of the government and the public, good conservation programmes are also needed. Such programmes require a thorough

foundation in population genetics and population ecology, areas which our curricula in colleges and universities typically neglect. One of the strategies for conservation of biodiversity should therefore be to sensitize educators for modifying the course curricula accordingly.

Conservation of biodiversity can be *in situ* as well as *ex situ*. In the former, forest departments establish national parks, protected parks, biosphere reserves and world heritage sites. *Ex situ* conservation is done by the government agencies and universities by setting up botanical gardens, zoological gardens and gene banks. Tribals, farmers and village communities also play very important roles both *in situ* on farm through land races and folk varieties and *ex situ* on farm through sacred groves.

The goal of a conservation strategy should be to ensure that evolution continues. Allowing for the play of natural forces by which both wild and domestic species evolve will maintain gene pools and retain genetic traits that may prove valuable in the future. Apart from protecting the species' capacity to adapt, it is important to preserve a constellation of species and genes for human needs we cannot anticipate how. Even when our food requirements become lower due to stability of population, diversity within a crop will suit human needs better. The availability of that diversity will depend on conservation choices made now.

The pristine South America's Galapagos islands where Charles Darwin spent five weeks in 1835 to collect material which led him to the famous theory of evolution by natural selection, are facing erosion of biodiversity due to the detritus of human development. Many of the islands' 1900 endemic species are threatened. The World Heritage site designated by the UNESCO on these islands has come close to being declared officially endangered. Benchley<sup>19</sup> describes the Galapagos as a paradise in peril. To prevent the depletion of the biodiversity, the Government of Ecuador passed a special law for the Galapagos in 1998 which puts restrictions on immigration, increases the percentage of tourist revenue going to the national park, expands the limits of the marine reserves around these islands and regulates the transport of introduced species.

In order to set up targets for conservation efforts, one should know the rate at which species are being lost as well as the manner in which ecosystems work. A new branch of biology, *conservation biology*, is emerging in which loss of diversity is studied in detail. The guiding discipline is, however, *conservation genetics* which studies the potential of species to survive and to evolve in parks and managed areas. In fact such areas should be maintained in their pristine purity to serve as nature's *evolutionary laboratories* where evolutionary mechanisms take place undisturbed. One can estimate

the size of an animal population that will preserve a desired amount of genetic diversity and foresee biological losses. For example, one study of the population genetics and ecological needs of large animals indicated that even the largest protected areas, without intensive management, are unlikely to sustain viable populations of predators such as wolves and mountain lions as well as large mammals including elephants. This virtually points out to their extinction in the wild within the next century. Recently a report in *Nature* indicated that the strategy for the conservation of endangered species should take greater account of the outer fringes of the geographical area in which the animal lives instead of just concentrating on core regions where the species is still most plentiful. It has been found that the giant panda, one of the endangered species, hangs on at the edges of its historical-geographical ranges instead of the central regions which would historically have provided the most favourable habitat. The recently extinct Tasmanian tiger became confined to the island of Tasmania in the extreme south of its previous range. According to ecological considerations, no species would choose life at the edges. It is the humans spreading across the face of a landscape previously populated only by wild animals, who have driven the species back to hang on wherever they can – in isolated mountain ranges and even on tiny islands – where human disturbance is less.

A little more than a quarter of the world's area in parks is a '*biosphere reserve*' – areas of special ecological importance designated as part of a worldwide network coordinated by the UNESCO's *Man and the Biosphere Programme*. Besides creation of parks and protected areas for conservation of living diversity, *in situ* gene banks should be established to complement the germplasm collections in which seeds and plant material are stored.

Since Darwin's time, it is known that species become rare before becoming extinct and that once rare, species are vulnerable to disappearance. One should not therefore ignore *rarity*. Future efforts to protect species and to prevent rare ones from slipping into extinction will depend on a deeper understanding of the biology of *rarity* and *extinction* and a sense of how human interactions with the biosphere affect them.

The cultural strengths of tribals in our country, who are the largest repository of living knowledge of forest diversity must be recognized, valued and harnessed for the continued preservation of diverse cultures and ecosystems. Agricultural diversity is a result of centuries of careful observations and innovative experiments by the farmers. The *Plant Varieties Act* should ensure that the basic contribution made by the farmers over generations of randomly fluctuating environmental conditions is recognized and made the basis of farmers' rights. Globally, 3.5 billion people depend on plant-based medicines. But even medicinal plants are getting extinct. The

wide use of the bark of the African cherry tree in Europe for treating prostate disorders has depleted this tree which grows in Cameroon and other certain African countries. The rich diversity of medicinal plants and associated traditional knowledge in India need therefore to be conserved and associated indigenous health care systems upgraded.

A computer-based and authenticated information system relating to the provenance, environmental ambience, characteristics and utilization of biodiversity should be organized and a *national information register* should be developed to prevent bio-piracy on the one hand, and encourage their continued utilization and upgradation by research and development on the other. In this connection, it is worth noting that a Global Biodiversity Information Facility (GBIF) is going to be created due to the efforts of a working group of the Megascience Forum, a body created by the industrialized country members of the Organization for Economic Cooperation and Development (OECD) to explore possible collaborations in building large scientific facilities. The GBIF will compile and disseminate information about the range of species on Earth. It will also coordinate the development of new software to link databases that embrace the full range of biodiversity information including geographical, ecological, genetic and molecular data. It will digitize all biodiversity information, now usually embodied specimens in museums, away from where the samples were collected.

### Measurement of species diversity

The assessment of biodiversity in a given ecosystem depends on making detailed inventories of species and varieties. This is a stupendous task. The National Academy of Sciences, USA has estimated that at least a five-fold increase in the number of systematists will be needed than at present to deal with a sizeable proportion of the diversity still existing, particularly in tropical forests. According to one estimate, out of the 10 million species existing, only 1.5 million have been described so far. The measurement of species diversity is essentially a statistical problem on which several research papers have been published. For instance, Fager<sup>20</sup> computed a *number of moves index* of diversity from a given sample on observed frequencies of a set of species in a given community which is scaled to give a value between 1 and 0. Patil and Taillie<sup>21</sup> suggested an *average variety index*, based on ranks, as a population diversity measure which increases with the addition of a new species. Lyons and Hutcherson<sup>22</sup> developed a large-sample distribution of the underlying statistics for the number of moves index of diversity and found it to be approximately normally distributed when species<sup>1</sup> fre-

quencies are asymptotically normal. However, for exact small-sample moments, Monte Carlo methods are used on high-speed computers.

With the advent of molecular genetics, data on diversity are being collected at an unprecedented rate by DNA sequencing. A global effort to survey genetic diversity among the world's human population is underway, led by Luca Cavalli-Sforza of Stanford University. The project, known as *Human Genome Diversity Project* (HGDP), envisages preparing cell lines and DNA from blood, hair or saliva samples taken from anonymous individuals in different populations across the globe, particularly those populations which have been geographically isolated or have a distinct culture and language. DNA sequences at a few dozen carefully chosen sites along the genome in individuals from every population will be compared. The resulting information – which sequence variables occur at each site in each population, and how prevalent each one is – can be used to infer degrees of relatedness and construct genealogical trees.

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