

existence and perpetuation of the first forms of life. Many of the cellular processes that are observed today, like RNA editing or intron splicing, they believe, are relics of the 'RNA world'.

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OPINION

Genetic resource mapping and fisheries management

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The Indian fisheries sector has witnessed two remarkable changes in the last four decades: (i) decline of natural fish populations in open waters¹, and (ii) development of aquaculture as a profitable business. Though fish production as a whole increased steadily during this period (0.7 million tonnes in 1951 to 4.5 million tonnes in 1993-94), the genetic and evolutionary consequences of these developments are less discussed and realized. We pointed out earlier² some genetically unsound practices which may affect aquaculture productivity and fish biodiversity. The effects of different anthropogenic factors on the fisheries resources are presented diagrammatically in Figure 1, which is self-explanatory.

In this article we emphasize the immediate and long-term importance of genetic resource mapping for safeguarding and utilizing more purposefully the fish biodiversity resources existing in India. We also elaborate here the concept of genetic resource mapping, the scientific approaches it needs, its approximate cost and utility.

Genetic resource mapping

Genetic variation in nature

Genetic variability occurs within and between the natural populations of a species occupying different geographical and ecological zones and is more prominent among freshwater fishes than in marine ones³. In population genetics terminology a 'stock' is defined as a randomly interbreeding, self-reproducing subset of a species that is geographically isolated from other such groups⁴. The genetic stocks (separate spawning populations)

that make up a species represent the fundamental units of both reproductive and genetic diversity; thus, they determine the ecological and evolutionary potential of the species.

The genetic variation among stocks could be correlated with some adaptive, physiological and behavioural changes in them. These changes include differential spawning time, growth rate, disease resistance, heat and cold tolerance, pH tolerance, migratory behaviour, spawning performance and catchability. Genetic diversity is associated with immediate fitness and long-term evolutionary poten-

tial⁵ and is also associated with enhanced mean fitness of fish populations⁶. Therefore, the stock concept has assumed tremendous importance for effective management and conservation of fisheries resources.

Conceptually, genetic resource mapping means identification and geographical localization of genetically differentiated populations (genetic stocks) of a species and assessing their evolutionary interrelationship by quantifying genetic identity/distance between them. In short, genetic resource mapping means documentation and cataloguing of intra-

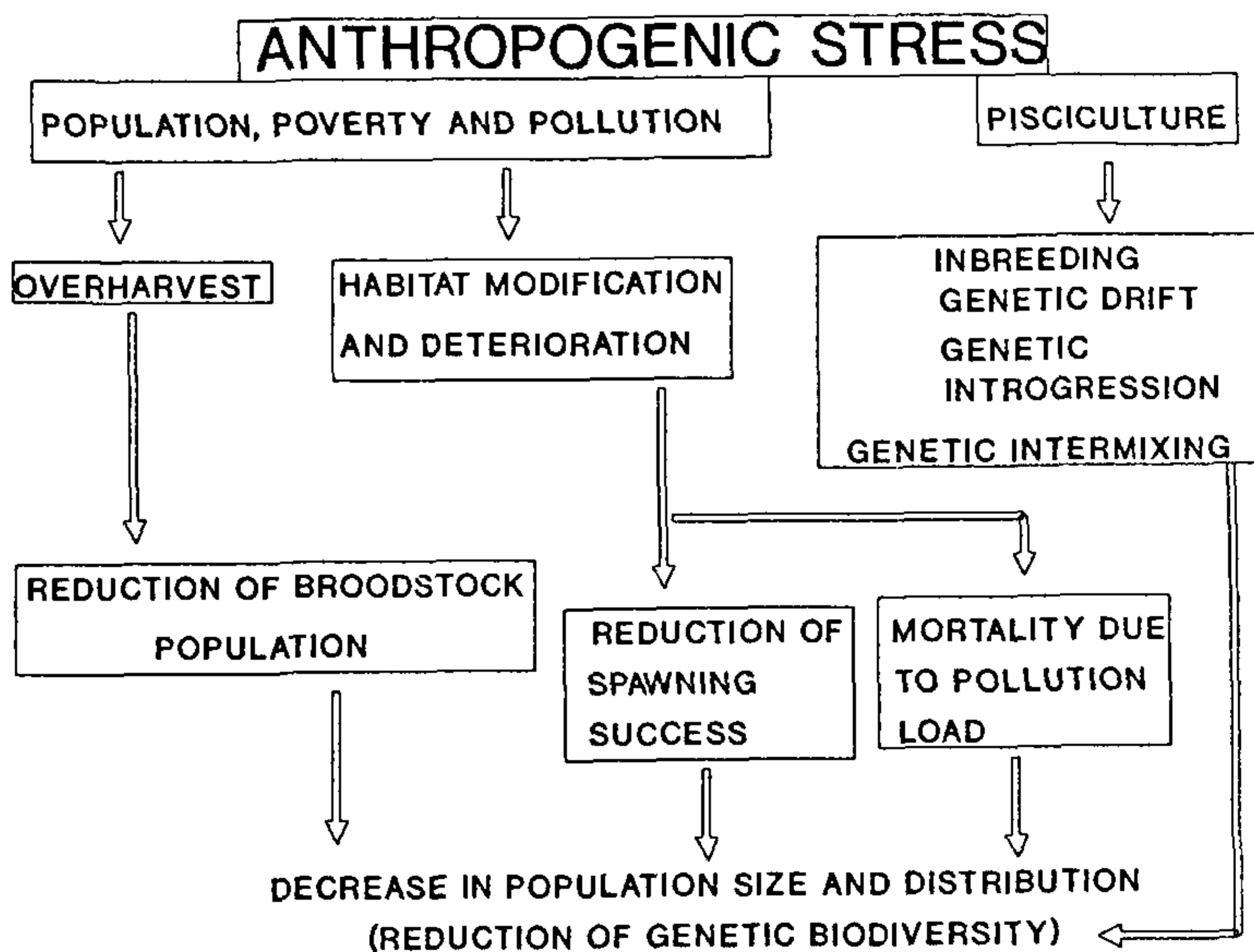


Figure 1. Anthropogenic factors operating in natural waters and aquaculture leading to the reduction of fish genetic biodiversity in the long run.

specific genetic biodiversity with an evolutionary bias. For drawing up of genetic resource map, aspects like molecular and biochemical genetics, evolution, zoogeography, ecology and demography of the stocks have to be taken into consideration.

Genetic stock identification

For genetic stock identification morphometric, meristic, cytogenetical, immunogenetical, biochemical and molecular genetic methods can be applied (Table 1). Of these techniques, biochemical genetic method, particularly the isozyme electrophoretic markers, was extensively applied for stock identification study in the recent past. Isozymes are the multiple molecular forms of an enzyme and are the direct gene products. Any detectable change in the isozyme phenotype reflects the genetic change at the DNA sequence. During the sixties and seventies isozymes in fish were extensively studied in some western countries and the knowledge was applied for fisheries management in the eighties⁷. Since isozyme pattern cannot detect point mutations and conservative amino acid substitutions, it reveals only one-third or less amount of the genetic variation actually present.

Rapid advances in molecular biology have helped to develop 'molecular markers' in the form of restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mt-DNA) and nuclear DNA (nu-DNA). Typically, RFLP relies on physical purification of DNA, cleavage with a series of restriction endonucleases and visualization of the resulting DNA fragments separated by electrophoresis. To locate the fragmenta-

tion pattern, labelled nuclear genes or oligonucleotide probes are needed. On the other hand, mt-DNA being rather small gives rise to only a limited number of fragments which can be directly visualized on a suitably stained gel after separation. However, due to poor yield or small sample size, labelling of the fragments can facilitate detection⁸. Mt-DNA RFLP is advantageous for its small molecular size (16–23 kb), higher evolutionary rate (than nu-DNA), lack of recombination, and predominantly maternal mode of inheritance. Therefore, mt-DNA RFLP came into application in fisheries science in the late eighties only in some developed countries. A similar study was recently initiated by us^{9,10}.

The advent of polymerase chain reaction (PCR) brought out some qualitative change in the approach of studying genetic variation in different populations of a species⁸. Specific genes can be amplified enzymatically from crude cell extracts using oligonucleotide primers corresponding to the two ends of a DNA (gene) fragment. Also, by using unequal amounts of two amplification primers, an excess amount of single-stranded DNA of a chosen strand can be synthesized for direct sequencing. By an appropriate choice of gene segments, it is possible to study DNA sequence variation among individuals, local populations and/or species¹¹. Genetic polymorphism in nuclear DNA can also be studied by using random oligonucleotide primers for PCR amplification, followed by gel electrophoresis. This polymorphism, called random amplified polymorphic DNA (RAPD)¹², can be used for genetic stock identification and also for identification of quantitative trait loci (QTL).

Applications

The scientific information contained in genetic resource map has varied practical utility.

Genetic stock integrity. Introduction of new stocks to an aquaculture system is relatively simple because such a water body is drainable and can be easily restocked. However, introduction of non-native stocks into large water bodies is problematic. Recently, the adverse effects on the fish gene pools arising due to genetic interaction between non-native/hatchery stock and the native/wild stock in natural waters after purposeful release of a non-native stock or its accidental escape from the aquaculture system were realized. For example the recent evidence of genetic interaction between farmed and wild Atlantic salmon (*Salmo salar*) in Irish rivers¹³ and introduction of Florida mouthbass gene into the native stock of the same species of Alabama public waters¹⁴ suggests the intermixing of genetically different fish stocks, leading to 'genetic homogenization' or 'admixture of genetic stocks'. Although hybridization (of different stocks) might provide a means of genetic diversity and fitness of hatchery stock, indiscriminate mixing could lead to outbreeding depression, a decrease in fitness attributable to negative interactions between differentially adapted genotypes⁵.

The interbreeding of different stocks leads to the formation of fertile F₁ hybrid. Subsequent inter-crossing of these hybrids with the parental population would lead to genetic introgression. As a result, contamination of the germplasm of the transplanted and the native stock would occur. This is undesired, since this phenomenon would severely hamper the breeding programme in future. The genetic stock integrity can best be protected provided we have the scientific database of our fish genetic resources.

Phylogeography and systematics. Genetic polymorphism often reveals geographical patterning¹⁵. This permits us to label a stock to its zoogeographical locality. Moreover, the intra-specific phylogeny study would enrich biosystematics. For example, estimates of variability within a species are necessary for estimating correctly the genetic distance among the species. Systematic record of intra-specific

Table 1. Techniques applied for stock identification

Technique	Markers scored	Degree of polymorphism
Morphometric measures	e.g. measuring body depth, head length	Low
Meristic counts	e.g. counting gill raker, fin rays	Low
Cytogenetics	Chromosome number and structure	Low
Immunogenetics	Antigenic determinants	Moderate
Isozymes	Amino acid sequence	Low
Nuclear DNA RFLP	Nucleotide sequence	Moderate/high
Mitochondrial DNA RFLP	Nucleotide sequence	Moderate
RAPD	Nucleotide sequence	Moderate/high
PCR-aided gene sequencing	Nucleotide sequence	High

RFLP = restriction fragment length polymorphism.
RAPD = random amplified polymorphic DNA

genetic variability would help to look for potentially misleading inter-specific hybridization or multiple-ancestral lineages which might be shared among species¹⁵.

Biodiversity assessment. The genetic stock identification in a species throughout its geographical territory would serve as an important guide for describing the biodiversity. For example, Bermingham and Avise¹⁶ found by mt-DNA RFLP analysis that the populations of each species of sunfish (*Lepomis*) and *Amia calva* inhabiting the freshwaters on the southwestern states of USA exhibit marked geographically concordant genetic differences. These differences were attributed to historical pattern of drainage isolation. These phylogenetic subdivisions served as an aid in the regional management programmes of these fishes. Moreover, screening of widely distributed populations of a given species would be useful in gaining an insight into the origin and evolutionary history of the species.

Stock rehabilitation and conservation. Fish populations in natural waters round the globe are declining due to over-harvest, pollution load, destruction of spawning ground and other man-made factors, depicted in Figure 1. In Indian waters the decline of carp, hilsa and mahaseer fishery has been recorded¹. The increased fish mortality in natural waters has demographic¹⁷ and evolutionary¹⁸ consequences. The demographic effects include decrease in size and age of individuals in fish populations, decrease in distribution and abundance of youngs, decline in catch per unit effort, etc. The evolutionary consequences would be the reduction of genetic diversity, which arose after thousands of years of the evolutionary selection process. The loss of genetic diversity is irreversible and the cost of the loss cannot be measured by any economic scale.

For framing a suitable strategy for conserving the declining and endangered stocks, three important questions are to be addressed: What to conserve? Where to conserve? How to conserve? If we have the genetic resource map, precise answers to the first two questions can be obtained. By periodic monitoring of the stock size and distribution, the valuable stocks that are declining can be identified and conserved. One approach for conservation is to catch the broodfishes of the

declining stock from natural waters, spawn them artificially and release them to their natural habitat. If the stocks are endangered, the genetic principles of conservation¹⁹ should be followed for stock rejuvenation. The site of conservation, whether it is a sanctuary or a hatchery or involves cryopreservation of gametes, can be decided. Thus, conservation planning and implementation can be more scientific.

Cryopreservation of gametes and embryo. Spermatozoa cryopreservation technology is an ingenious tool for the management of fisheries resources and can be applied (i) to rehabilitate an endangered fish species, (ii) to conserve the intra-specific genetic diversity of species, (iii) to cross the different stocks of a species conveniently by carrying the sperm from far-off places, and (iv) to avoid the difficulty of artificial breeding owing to non-synchronous maturity time of two different sexes of a species. However, the cryopreservation technology can be utilized effectively provided population genetic research in fish moves ahead in assaying the intra-specific genetic diversity. The same applies also to egg and embryo cryopreservation, which are just being attempted in fish.

Aquaculture. Some constraints of aquaculture productivity are of genetic nature². For example, inbreeding leads to fry deformity, decreased food conversion efficiency and reduction in growth rate. Once the stocks are characterized, the broodstocks of different genetic background can be selected and interbred to maximize heterozygosity. Thus, crosses between the individuals of the Atlantic salmon from different populations and the early maturing and late maturing stocks of rainbow trout revealed heterosis with respect to growth rate²⁰.

Cost and time scale

Considering the importance of genetic resource map, we strongly recommend a concerted scientific effort in this direction. Biodiversity assessment in commercially important fishes like Indian major carps and catfishes like *H. fossilis* and *C. batrachus* should be taken up immediately in our country. The samples of different populations should be collected from natural sources, then analysed through

biochemical and molecular biology techniques. The zoogeographical, ecological and demographical data should be recorded as far as practicable. Initially, the fish samples should be collected from natural sources at some major spots and their population genetics studied. If one or two strongly motivated groups (5–10 heads) work consistently for five years, at an approximate cost of Rs 50 lakhs (excluding the infrastructure they can make a crude genetic resource map of a few species, say three species of IMCs. We believe that the cost is quite nominal and affordable in considering the potential benefits of such a study.

Concluding remarks

Effective conservation and management efforts require well investigated scientific data. A genetic resource map would be one such data-bank that can help the planners and resource managers in conserving the vast and varied but dwindling fisheries resources of our country. Pandian *et al.*²¹ have pointed out the need of molecular biological study in fishes, particularly for developing transgenic lines. It is our concerned opinion that the horizon of molecular biology research in fish needs to be extended to genetic resource mapping.

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ACKNOWLEDGEMENTS. Thanks are due to Mr. Sudip K. Ghosh for help. B. K. P. thanks CSIR, New Delhi, for a fellowship

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Endemic, rare and threatened flowering plants of South India

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Endemism in plants is demonstrated at various geographical scales. A plant species can be restricted to a continent, region or even a locality. Gentry¹, while discussing endemism in neotropical plants, identified four forms, viz. island endemism, relict endemism, neoendemism and anthropogenic endemism. Island endemism is the most common form; as it does not concern us in the present context, we may ignore it. Relict endemism is represented by an ancient taxonomic lineage or distribution restricted in range by specialized habitats which are themselves result of past geological events. Species of plants in the genera *Mahonia* and *Rhododendron* in the hilltops of Western Ghats are good examples of relict endemism. The assumption, nevertheless, is that these plants were widespread in the past when the prevailing environmental conditions were more favourable.

Neoendemism are newly evolved plants which are usually restricted to the site of origin, characterized by the local occurrence of a number of closely related species. The many localized species of *Nilgirianthus* (Acanthaceae) in the hills of south India are examples of neoendemism.

Anthropogenic endemism is more a result of recent human destruction of habitats, exterminating some species locally. There may be a number of species qualifying for inclusion under this category of endemism. At least one ex-

ample, *Aponogeton appendiculatus*, which is currently endemic to Alapuzha in Kerala, is also known from Madras². The species certainly occurred in many tanks in the intervening area till recently.

Besides the four forms of endemism discussed above, there is in our opinion a unique fifth form, viz. pseudoendemism – plants which are apparently endemic due to an inadequate knowledge of their geographical ranges. It is rather compelling to believe that a large proportion of local endemics, at least in south India, is due to lack of intensive surveys of plant species in their natural habitats. An example supporting this pattern is of *Piper barberi*, listed by Nayar and Sastry² as being endemic to Kanyakumari district, which we have discovered in Kulathupuzha (Quilon). A detailed discussion of endemic south Indian flowering plants may be found elsewhere³.

Though rare plants have been the cause of much concern to conservation biologists, there has not been a single general definition of rarity. Rabinowitz *et al.*⁴ have suggested that rarity in plants can be assessed by their geographical range, habitat specificity and population size. A species of plant (e.g. *Terminalia paniculata*) that is most widespread and occurring in all available habitats is not rare. However, on the other extreme, we might find a species (e.g. *Elaeocarpus blascoi*) both localized and restricted by habitat. Such plants can certainly be considered rare. In this spectrum of rarity

there are included two other categories, viz. species with wide ranges though with restricted habitat preferences (e.g. *Pinanga dicksoni*) and species with restricted geographical ranges but without any specialized habitat preferences (e.g. *Anaphalis* spp.) If information on the population sizes is also available, we may further divide each of the four categories discussed above into those existing in small populations everywhere and those having a large population somewhere over their ranges. The eight rather discrete forms of rarity which thus emerge can be used while assessing the status of plant species⁴.

Studies in the neotropics have indicated that whereas endemism and rarity of species are often independent¹, certain families or genera do have more endemic as well as rare species than others⁵. It is probable that such families or genera diversified locally and recently¹ or they share some common traits that render them rare⁶. It thus becomes apparent that endemism and rarity in plants are a result of (i) biogeography, (ii) phylogeny and (iii) ecology (including anthropogenic influences).

Currently, in India, the M. S. Swaminathan Research Foundation with the cooperation of the Tamil Nadu State Forest Department is making an attempt at outlining a conservation strategy for the endemic, rare and threatened plants of the state. For this purpose, we selected the flowering plants of Tamil Nadu listed