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CONSERVATION GENETICS, COMBINATION OF MOLECULAR BIOLOGY AND ECOLOGY: TROPICAL TASAR SILKWORM AS AN EXAMPLE

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Biodiversity Conservation

Habitat fragmentation

Molecular markers

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ABSTRACT

In recent decades due to anthropogenic activities like pollution, habitat destruction and over-exploitation etc. there is a constant threat to biological diversity. Habitat loss poses the greatest threat to the long-term survival of many species on earth. Habitat fragmentation causes decrease in species richness, increase the edge effects, decrease in the density and abundance of species, alteration in inter-specific interactions and ecological processes.The genetic diversity of populations responds to environmental heterogeneity via alterations in the relative strengths of the four opposing genetic forces: mutation, migration, selection, and genetic drift. The balance and cumulative history of these forces determines actual levels of genetic diversity at any one time. While analyzing the conservation status of a species, the genetic diversity, gene flow, heterozygosity, and population structure are the essential parameters that need to be assessed for population under habitat stress. These parameters can be precisely analyzed through the use of molecular markers like RAPD, ISSR, SSR, AFLP etc. aided with mathematical calculations. Simultaneously, the ecological data are also taken into consideration. For conservation and restoration genetics of species under stress, molecular ecology approach is very appropriate. At the same time, the genetic status of any indicator species also speaks about the status of the habitat as a whole. Economically important wild tasar silkworm *Antheraea mylitta* Drury once upon a time flourished over entire tropical belt of India but now remains confined to limited ecopockets with patchy distribution. The conservation genetics has been worked out in such type of populations through molecular a marker which speaks of the local extinction process. The present paper deals with the molecular biology approach in conservation genetics cited by the example of tropical tasar silkworm as an indicator species.

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INTRODUCTION

During the last century, many species on the earth faced tremendous pressure of extinction due to different human activities like increase in pollution, habitat destruction, over-exploitation etc. The habitat fragmentation events resulted in reduced population size and deteriorated the conditions for long-time survival of several species including the wild sericigenous insects. The area of conservation biology, concerning preservation of biodiversity, has therefore been a huge and rapidly growing discipline of research in the last decades.

Genetic diversity is an essential aspect in conservation biology. The fundamental theorem of natural selection tells us that ''the rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time'', *i.e.* the rate of evolutionary change in a population is proportional to the amount of genetic diversity available. Consequently, loss of genetic diversity reduces future evolutionary options. Decreasing genetic diversity increases the extinction risk of populations due to a decline in fitness of individuals, which has been endorsed by many authors (Frankham, 1995; Bouzat *et al.*, 1998; Steffan-Dewenter and Tscharntke, 2002; Polus *et al.*, 2006). Genetic diversity is inversely proportional to isolation and that it is directly correlated with population size. The genetic diversity of populations responds to environmental heterogeneity via alterations in the relative strengths of the four opposing genetic forces: mutation, migration, selection, and genetic drift. The balance and cumulative history of these forces determines actual levels of genetic diversity at any one time.

Habitat Fragmentation And Population Genetics

Habitat fragmentation, *i.e.* the reduction of continuous habitat into several smaller spatially isolated remnants, decreased area, increases edge effects, alters ecological processes, and decreases connectivity (Debinski and Holt, 2000). Ecological studies of species distributions under fragmented conditions have generally shown that species with varying habitat requirements and life history traits respond differently to habitat fragmentation. Species with small populations and specialized habitat tend to decline while generalist and opportunistic species may be unaffected or thrive. This difference may be caused by a greater reduction in suitable habitat for specialists and a greater reduction in connectivity among populations if specialists cannot disperse through the surrounding habitat.

Populations on isolated habitat patches are expected to exhibit several genetic characteristics. Estimates of genetically effective population sizes are expected to be small in isolated populations and this will enhance the effects of genetic drift. Genetic drift reduces the number of alleles in populations and decreases mean individual heterozygosity. Thus, isolated populations that experience drift are expected to be deficient in the frequency of heterozygous individuals relative to Hardy-Weinberg expectations and to have elevated inbreeding coefficients. The genetic structure of populations will also be affected by habitat patchiness. Gene flow between populations will be reduced in a fragmented landscape leading to population differentiation. Recently isolated populations may show isolation-by-distance in which there is a negative correlation between geographic distances between populations and their genetic similarity. Population isolation leads to genetic differentiation such that more geographically separate populations are expected to be the most divergent genetically.

Both theoretical and experimental studies have outlined patterns of genetic differentiation expected from habitat fragmentation (Lande and Barrowclough, 1987; Templeton *et al.*, 1990; Frankham, 1995; Harrison and Hastings, 1996; Templeton, 1998, Spencer *et al.*, 2000). First, populations in fragmented habitat may experience restricted gene flow among populations, resulting in higher levels of genetic differentiation among populations. Second, isolated populations may be more likely to experience population bottlenecks, which in turn leads to reduced genetic variability (Frankham, 1995; Bouzat *et al.*, 1998; Westemeier *et al.*, 1998). A reduction in genetic variability is likely to be manifested as a reduction in allelic diversity (Spencer *et al.*, 2000). Also, both expected and observed levels of heterozygosity should be lower in bottlenecked populations and both heterozygosity levels will vary depending on the severity and length of the bottleneck, as well as the mating system and life history characteristics of the species.

In recent years there has been many studies on genetic effect of habitat fragmentation. Some studies examined the effects of natural, long-term fragmentation (Cunningham and Moritz, 1998; Barratt *et al.*, 1999; Clark *et al.*, 1999; Seppa and Laurila, 1999; Bates, 2000; Wolf *et al.*, 2000; Vucetich *et al.*, 2001) while some other studies focused on recent, anthropogenic habitat fragmentation at relatively small geographical scales (Gaines *et al.*, 1997; Peacock and Smith, 1997; Aldrich *et al.*, 1998; Gibbs, 1998; Van Dongen *et al.*, 1998; Dayanandan *et al.*, 1999; Gerlach and Musolf, 2000; Knutsen *et al.*, 2000; Mossman and Waser, 2001). The most commonly observed results from studies of habitat fragmentation are at significant levels of differentiation among populations, and low levels of genetic variation within populations, relative to related taxa (Gaines *et al.*, 1997). Low gene flow species may also be more likely to experience local adaptation to a given area and, consequently, are less prone to changes resulting from habitat loss and fragmentation.

Molecular Markers In Measurement of Genetic Diversity

The interest has also increased recently in the use of DNA-based markers for a variety of applications in population genetics, conservation and crop improvement. They can potentially provide a much higher number of markers than can allozyme analysis. Moreover, unlike the morphological or phenotypic markers, the DNA based markers are free from environmental influence and developmental stage of the organism under study. Among the different DNA-based methods, PCR-derived markers obtained with unspecific primers have become exceedingly popular for estimations of within-species variability. Since no sequence information for the target species is required, these methods are especially suited to situations where little or no molecular genetics research has been conducted previously. Molecular markers have been widely used in studies of the genetics of wild populations. The technique enables researchers to answer questions about speciation, genotypic diversity within and between populations, gene flow characteristics and levels of outcrossing. Molecular markers provide new tools for ecological and genetic studies of evolutionary processes. Newer markers such as microsatellites, inter-simple sequence repeats (ISSR) and random amplified polymorphic DNA (RAPD) provide more detailed genetic information due to either the increased variability of loci or the greater numbers of loci available. Among the other molecular techniques, amplified length polymorphism (AFLP), simple sequence repeats (SSR) or microsatellites, single nucleotide polymorphism (SNP), mitochondrial DNA, chloroplast DNA etc. are presently utilize to the genetic diversity and genetic structure of different plant or animal populations under ecological stress. The mathematical foundations for measuring and interpreting genetic diversity and population genetic structure were developed in the early part of the last century. However, progress in understanding genetic processes in populations was limited until the explosion of molecular biological methods made it possible to accurately apply and test genetic theory in the field. Different types of molecular markers described above have been effectively utilized in analyzing the genetic diversity and phylogenetic relatedness in sericigenous insects (Arunkumar *et al.*, 2006), *Antheraea mylitta* (Chatterjee *et al.*, 2004; Kar *et al.*, 2005; Saha and Kundu, 2006; Mahendran *et al.*, 2006) and even the domestic silkworm *Bombyx mori* (Hou *et al.*, 2007).

Genetic status of populations under ecological stress

One of the advantages of genetic diversity measures is that they are highly scalable indices. An enhanced ability to describe patterns of genetic diversity in space (spatial) and time (temporal) has, in turn, made it easier to diagnose the genetic mechanisms that produce these patterns (Carvalho, 1988). The parameters that characterize populations are gene flow, migration rates, effective size, change in size, relatedness and phylogeny. By far, the easiest genetic forces to measure in natural populations are equilibrium levels of migration (gene flow) and genetic drift. Gene flow provides a direct measure of the evolutionary connectivity of populations while genetic drift provides a measure of effective population size. Populations that have low connectivity with others become genetically differentiated and more unique. These populations are often assigned higher conservation 'value' because a large portion of the genetic diversity of the species would be lost if they were extirpated (Moritz, 1995). Populations with low effective population size may be more vulnerable and therefore may be targeted for immediate conservation efforts.

Measures of genetic diversity, such as Wright's F statistics (F_{ST}) or Nei's genetic diversity statistics (Nei, 1987) are especially appropriate for analyzing this hierarchical structure. These population statistics can be used to assess the proportion of all genetic diversity that resides within populations, within local habitat, within the larger landscape and within regions. Analysis of molecular variance technique (AMOVA) is a powerful tool to analyse marker variation within and among populations under consideration.

There exists a clear relationship between genetic diversity and ecological stressors. In general, different stressors have predictable consequences for measurements of genetic diversity. This is not to say that genetic diversity is likely to be useful to diagnose specific stressors, but that an established cause and effect relationship exists. Loss of habitat that causes a decrease in the rate of migration between populations (fragmentation) is expected to increase the component of genetic diversity among populations, which may be measured as a decrease in the average similarity between populations or an increase in estimates of one of Wright's F-statistics (Wright, 1978), F_{ST}. If fragmentation results in small breeding population sizes, then a second predicted outcome would be reduced within-population genetic diversity or heterozygosity (H), and reduced numbers of segregating alleles per locus (Na). The predicted effect of introduced species depends on whether they can successfully hybridize with native populations. Thus, introduced species (or populations) are expected to have an effect similar to habitat degradation (decreased H, Na) due to competition or predation but will increase genetic diversity through hybridization.

Indicators to evaluate ecosystem health

To assess the health of a habitat, one of the most important potential uses of a genetic diversity indicator is simply to characterize the natural population structure of component populations within ecosystems. The effective population size and connectivity of populations are fundamental attributes of populations that can be diagnosed only with genetic markers as described above. Genetic diversity responds to environmental change, primarily through the actions of genetic drift and selection, but also through mutation and gene flow. The indicator 'remembers' population effects. In other words, once genetic diversity is reduced it will remain low until mutation or gene flow replenishes it. Thus, a genetic diversity indicator is expected to be useful primarily for multigenerational exposures; the relevant timescale for indicator response is years or decades, not months. Exceptions occur when populations are severely bottlenecked or severe selection pressures are imposed on populations, inducing rapid and severe changes in genetic diversity. The effects on anthropogenic stresses leading to local extinction are presented in Fig. 1 and the genetic parameters can be evaluated specifically to assess the conservation status of populations. Through this the effective restoration genetic measures can be taken in due course**.**

In many parts of the world, Lepidoptera are widely accepted as ecological indicators of ecosystem health (Rosenberg, 1986; New *et al.*, 1995; Beccaloni and Gaston, 1995; Oostermeijer and vanSwaay, 1998), and meet a number of the criteria set forth by Hilty and Merenlender (2000). Lepidopterans in general and butterflies in particular were used as models because (i) the amount of modifications in their community structure and composition is proportional to the degree of fragmentation (Thomas, 1991, New, 1997) (ii) they react quickly to environmental changes (Erhardt and Thomas, 1991; Bourn and Thomas, 2002) and (iii) they are considered to be umbrella species (sustaining habitat to conserve them will also conserve many other taxa) and indicators of habitat quality (New, 1997). Specialist (monophagous) butterfly species are more affected by environmental changes than generalist species (Steffan-Dewenter and Tscharntke, 2002; Polus *et al.,* 2006). It has been clearly demonstrated and confirmed theoretical predictions that a decrease in habitat area leads to a decrease in species diversity and abundance and to an alteration of Lepidopteran community composition (Vandewoestijne *et al.,* 2005).

In Indian context there has been little study on the effect of habitat fragmentation or any environmental stressor on the biodiversity utilizing any indicator species. Kar (2009) has recently studied the genetic diversity and genetic structure of selected wild populations of tasar silkworm in Simlipal Biosphere Reserve utilizing molecular markers. There has been sufficient evidence of habitat fragmentation obtained from the genetic structure (F_{ST}), gene flow (Nm), heterozygosity (Ht), analysis of molecular variance (AMOVA) of

these populations in a discontinuous habitat. Initial attempt has been made to utilize such a species to assess the habitat health of this biosphere reserve and conservation measures are suggested. Lepidopteran species can be used as ideal indicator for assessment of habitat health through above said means and appropriate strategy can be formulated for the conservation of biodiversity available in the country not only for the vulnerable populations but also for sustainable utilization of genetic resources. Effective conservation strategies of wild silk moths are to be chalked out with a strong genetic basis for pre and post evaluation of conservation efforts.

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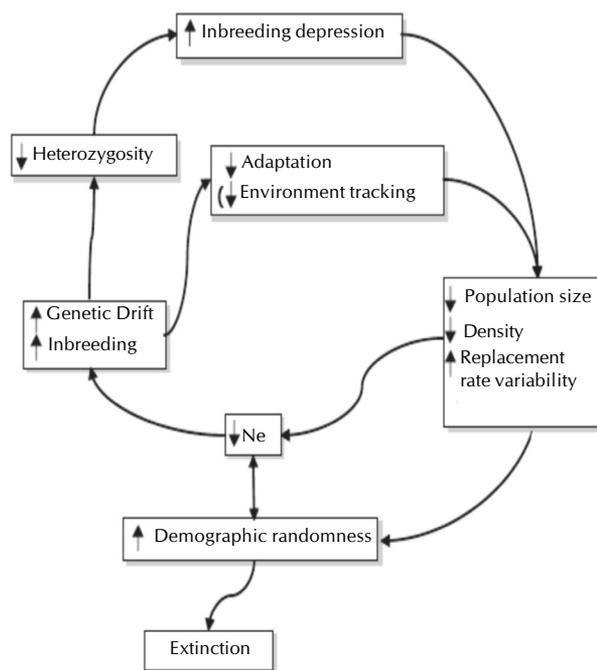
Figure 1: Genetic factors leading to the local extinction of species Ne – Effective population size (adapted from Gilpin and Soule, 1986)

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