Table 1. Different indices to comment on the species structure of the study sites

Study sites	Birds site	Species site	Richness index	Diversity index	Evenness index	Dominance index
RmC	59	29	6.87	5.26	1.29	0.16
SiC	56	27	6.46	5.11	1.55	0.19
NgC	52	26	6.34	4.95	1.52	0.18
KeC	43	25	6.38	4.42	1.37	0.15
KuC	32	16	4.34	3.94	1.14	0.30
BvC	78	40	8.95	5.75	1.56	0.11
EpR	75	42	9.50	5.49	1.47	0.09
EgC	77	40	8.98	5.69	1.54	0.10
AnR	73	36	8.16	5.70	1.59	0.13
mean	60.5	31.2	7.33	5.15	1.40	0.126
SD	15.4	8.25	1.58	0.58	0.15	0.05
Lower reaches	320	78	13.35	4.56	1.05	0.007
Higher reaches	225	83	15.14	4.70	1.06	0.009

further observational support. Absence of relatively common species of foothills, peripheral jungles and around habitations in the study sites and alongside the occurrence of very rare Mishmi Wren-babbler and many other less common species also demands further confirmation and more intensive studies. Some of the species, though recorded as rare in the present study, might appear regularly during other seasons or if the study was more intensive. The short list of species under the families, Sylviidae, Prunellidae and Dicaeidae was due to the limitations in study duration. However, the present effort records, for the first time, the avian assemblage of Mouling National Park with comments on their community structure. This study would help in the initiation of more detailed studies to enrich the list of species and to record the nature and extent of passage and local migration with the resident species.

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Molecular markers for genetic fidelity during micropropagation and germplasm conservation?

In recent years, with the advent of recombinant DNA technology and PCR, molecular markers are being used for a variety of studies. The molecular markers include Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) or Sequence Tagged Microsatellite Sites (STMS), Sequence Tagged Sites (STS), DNA Amplification Fingerprinting (DAF) and Microsatellite Primed-PCR (MP-PCR) (ref. 1). More recently, molecular markers have also been used for testing the genetic fidelity during micropropagation/*ex situ* conservation on the one hand, and for characterization of plant genetic resources on the other. This aspect of the use of molecular markers has received attention in recent years due to the significance that is being attached to micropropagation of elite genotypes and to the *in situ* and *ex situ* conservation of plant genetic resources (PGRs). Molecular markers have particularly been suggested to be useful for confirmation of genetic fidelity in micropropagated tree species, where life span is quite long and performance of micropropagated plants could only be ascertained after their long

juvenile stage in field conditions. In India also, studies have been conducted and research projects funded for research where lack of polymorphism shown through the use of molecular markers has been used to infer genetic fidelity[2,3].

In commercial industry, where micropropagation technology has been developed, their foremost concern is the maintenance of true-to-type nature of the micropropagated plants *vis-à-vis* explant source. This can be achieved by developing the micropropagation protocols based either upon axillary branching or somatic embryogenesis. These two methods are believed to give rise to genetically uniform and true-to-type plants, since the organized meristems do not undergo genetic changes that might arise during cell division or differentiation from callus cultures[4,5]. Although a number of reports support this view[6–8], there are some other reports also which document the occurrence of somaclonal variation even among plants derived either through somatic embryogenesis or through enhanced axillary branching cultures. It has, therefore, been suggested that commercial application of tissue culture to perennial crops must await adequate quality check under field conditions irrespective of the method used for micropropagation[9]. However, if the analysis is carried out during various cultural passages; it would establish genetic variation (if any) very early in the cultural systems, so that one may like to suitably modify the micropropagation protocols to avoid the above variation.

Several cytological, isozyme and molecular markers have been used to detect the variation and/ or confirm the genetic fidelity in micropropagated plants[3]. A large number of RFLPs were recorded in regenerated maize, rice, wheat, barley, triticale, potato[3] and some tree species including populus, eucalyptus and coffee[1] for studying the variation and genetic fidelity during micropropagation. Brown *et al.*[10] also suggested the use of PCR in the analysis of tissue culture-derived plants, so that PCR has been used specially in the form of RAPDs in onion[11], *Picea*[12], populus and eucalyptus species[2,3] and in the form of MP-PCR in eucalyptus, populus and coffee species[3].

In several reports, variations have been observed through molecular markers and characterized as somaclonal variation. For instance in rice, doubling the time of *in vitro* culture resulted in quadruplicating the detected DNA polymorphisms in regenerants[13]. However, in some other studies, the lack of polymorphism in micropropagated plants screened through molecular markers was used to suggest genetic fidelity. For instance, in a study on *Picea* the genetic integrity during somatic embryogenesis has been studied using RAPDs[12]. In India also, an extensive study on genetic fidelity and molecular diagnostics in micropropagation systems was carried out where several molecular markers including RFLPs (using rDNA probes and mtDNA probes), RAPDs, MP-PCR and oligonucleotide in-gel hybridization were used in micropropagated clones of 4 tree species namely *Populus deltoides, Eucalyptus tereticornis, E. camaldulensis* and *Coffea arabica*[3]. In this study, the authors inferred genetic fidelity in those micropropagated clones where molecular markers failed to detect any polymorphism. In their study, the micropropagated clones of *P. deltoides*, when examined for RFLPs, using three heterologous rDNA probes, showed uniformity in combination with 6 base cutter enzymes but the same clones revealed distinct variation using 4 base cutter enzymes. A reverse situation was observed in *Coffea arabica*. It is apparent thus, that sometimes, the same clones may show uniformity with one molecular marker and variation with another marker. In further studies, while using a set of 25 RAPD primers, 4 out of 5 clones of *P. deltoides* and all the clones of *E. tereticornis* and *E. camaldulensis* showed uniformity. The use of 5 oligonucleotides complementary to microsatellites namely $(GATA)_4$, $(GACA)_4$, $(GTG)_5$, and $(TCC)_5$ in MP-PCR and in-gel hybridization also showed uniformity. We, however, believe that the use of a larger number of RAPD primers, additional microsatellites and other molecular marker systems could detect the variation in those clones also where uniformity was observed with a limited number of primers or oligonucleotides/enzyme combinations as above. Such a belief is based on our own experience in bread wheat, where failure of the detection of polymorphism by a molecular marker system indicated the inadequacy of the molecular marker for detecting polymorphism, so that relatively more powerful molecular markers need to be used to detect polymorphism. In our own studies, different molecular markers, namely oligonucleotide in-gel hybridization, RAPDs, MP-PCR, DAF, STS and STMS, have been used in 10 bread wheat genotypes. The use of as many as 23 SSR probes in combination with 14 restriction enzymes (a total of 142 probe-enzyme combinations) failed to detect the polymorphism in these genotypes[14]. Our unpublished preliminary results on RAPDs, MP-PCR and AFLPs also showed lack of polymorphism in these genotypes. But this does not mean absence of polymorphism in the material studied but instead suggested inadequacy of these marker systems, since other molecular markers (e.g. DAF, STS and STMS) did detect adequate and reproducible polymorphism in the same material[15–17]. We, therefore, are of the opinion that any failure to detect polymorphism should not be used to infer genetic fidelity. We also like to emphasize that each marker system screens only a fraction of the genome and not the whole genome and that different markers may screen different fractions of the genome. The entire genome cannot be studied on the basis of only one type of molecular marker. For instance, the oligonucleotide in-gel hybridization is only suitable for studying the repetitive DNA[18]; RFLPs are suitable only for the study of variation in restriction sites of a particular restriction enzyme. In view of this, any molecular marker system needs to be first assessed for its suitability for DNA fingerprinting and for ascertaining genetic fidelity.

Molecular markers have also been used to find out whether or not additional variation originates during storage and conservation, (i.e. detect duplications, seed mixtures, inadvertent outcrossing and genetic drift). For this purpose, our experience suggests that DNA fingerprinting using microsatellites, DAF and AFLP markers may be relatively more suitable. Microsatellite markers have been used successfully to determine the degree of relatedness among individuals or groups of accessions, to clarify the genetic structure, or partitioning of variation among individuals, accessions, population and

species of rice[18]. RFLPs (using nDNA and cpDNA probes) and RAPDs were also used for characterization and identification of genetic resources of perennial crops like *Musa* and to solve problems related to plant genetic diversity conservation[19]. The potential of molecular markers have also been shown in other crops for characterization and identification of core collection for germplasm conservation[20]. For germplasm conservation also, we believe that individual molecular markers, unless proved to be suitable for DNA fingerprinting, should not be used to study the genetic fidelity but only for detection of genetic variation arising during storage and conservation.

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