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Are Small Forest Fragments More Heterogeneous among Themselves than Large Fragments?

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ABSTRACT

In the present study of shola forest fragments of central Western Ghats, India, two predictions of Ganeshaiah *et al*. (1997) were tested: (a) whether the gene assemblages among a set of larger forest fragments are more similar to each other than among a set of small fragments? and (b) are the coefficient of variation for the similarity in the genetic status of population higher for smaller than the larger forest fragments? However, whereas Ganeshaiah *et al*. (1997) observed the evidence from species assemblages, we looked at the evidence from the genomic DNA of a single widespread tree species, *Litsea floribunda* Gamble, found abundantly in all the shola fragments of central Western Ghats. We argue that just as a set of co-adapted species might be selected in an island, a set of co-adapted genes in a species may also be selected in an island. By studying the RAPD-PCR amplification products in the populations of *L. floribunda* Gamble we tested our prediction. Our results show that populations in small forest fragments tend to be more genetically dissimilar to each other than do large fragments among themselves. These results have important implications for the conservation of genetic resources in fragmented habitats.

Keywords: genomic DNA, island biogeography, *Litsea floribunda* Gamble, Shola fragments, similarity index, species assemblages
Abbreviations: BRTWS, Biligiri Rangan Swamy Temple Wildlife Sanctuary; **BWS**, Brahmagiri Wildlife Sanctuary; **CV**, coefficient of variation; **PCR**, polymerase chain reaction; **RAPD**, randomly amplified polymorphic DNA; **SEM**, standard error of the mean

INTRODUCTION

Fragmentation of natural habitats into smaller and non-contiguous patches is the most serious threat to the long-term survival of the biological diversity on earth (Myers 1994; Chapin *et al*. 2000; Pimm and Raven 2000; Cruse-Sanders and Hamrick 2004). In the last few decades, there has been a growing concern over the alarming rate of deforestation and habitat fragmentation of the once pristine tropical forests. In India, the rate of deforestation has been estimated to be in the order of 0.57% annually (Menon and Bawa 1998; Uma Shaanker *et al*. 2004). A far more distressing feature is that most of the forests that have been left behind are highly fragmented. In the Western Ghats of India, one of the richest biodiversity hotspots of the world (Myers 1994), it was estimated that about 25.6% in forest cover was lost in the last two decades owing to the varied spatial variability in the land use pattern. This has resulted in the increase of degraded forest by 26.64% (Jha *et al*. 2000). It has also been recorded that there has been a substantial increase in the forest patches (from 179 to 769) in the Western Ghats (Menon and Bawa 1998; Uma Shaanker *et al*. 2001, 2004). These fragmented patches of forest are often embedded in a matrix of anthropogenically manipulated landscapes (such as pastures, agricultural fields or habitations) and behave as "islands" in a "sea" of pasture or agricultural ecosystem.

Such insularized forest fragments could lead to distinct ecological, demographic and genetic consequences and might occasionally result in local or global extinction of the native species (Gilpin 1988; Tilman *et al*. 1994; Gascon *et al*. 2000; Laurance 2000; Pimm and Raven 2000; Young and Boyle 2000; Nageswara Rao *et al*. 2001; Uma Shaanker *et al*. 2001; Ravikanth *et al*. 2002; Cruse-Sanders and Hamrick 2004; Honnay *et al*. 2005; Nageswara Rao *et al*. 2007). These consequences can often be traced to the underlying genetic changes in the populations such as loss of genetic diversity and an increased level of inbreeding depression. Genetically depauperate populations are less likely to respond to the selective pressure of a changing environment and may be more likely to suffer extinction under conditions of continued fragmentation (Franklin 1980; Beardmore 1983; Simberloff 1988; Young and Boyle 2000). Restricted gene flow among islands and their species gene pool could facilitate inter-island evolutionary divergence and could increase endemism (Hamrick and Schnabel 1985; Boshier *et al*. 1995; Wu and Vankat 1995; Nageswara Rao 2004).

Previous studies have been carried out to assess the impacts of fragmentation on the genetic consequences in populations (Kruckeburg and Rabinowitz 1985; Wicknes-wari and Boyle 2000; Nageswara Rao *et al*. 2001; Padmini *et al*. 2001; Ravikanth *et al*. 2001; Uma Shaanker *et al*. 2001; Nageswara Rao *et al*. 2007). However, these studies focused only on forest fragments that were recent in their origin and dealt with anthropogenically caused fragmentation, whose history of existence had been short (Honnay *et al*. 2005). One of the limitations from these studies is that they mostly reflect short-term consequences immediately after the fragmentation events. To rectify this, populations of species in more stable forest fragments or islands with a long history of existence offer an exciting model system to investigate fundamental questions on forest fragmentation. It would be useful to look at the genetic diversity para-

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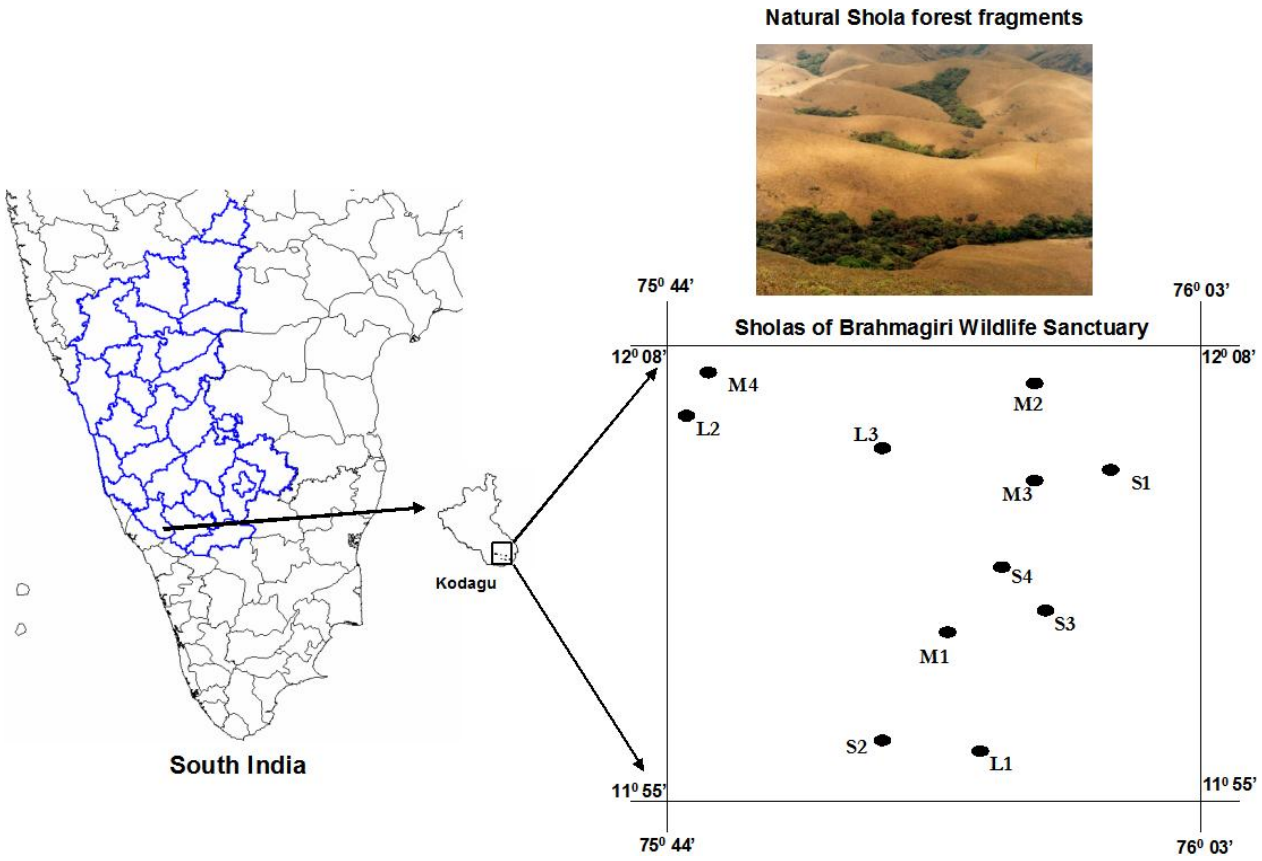

Fig. 1 Distribution map of study sites (sholas) in Kodagu district, central Western Ghats, India. The photograph on the top right-hand corner depicts a typical high altitude evergreen forests which occur in the valleys and slopes of hills in discrete patches set amidst a grassland landscape found in the Western Ghats, India. S = small, M =medium and L = large sized shola fragments.

meters of species in long lived fragments or permanent fragments which might have attained equilibrium status both at species and at genetic level. In the theory of "island biogeography", MacArthur and Wilson (1967) proposed that islands attain equilibrium with respect to the number of species they can harbor (Assortative Equilibrium Status). However, Ganeshaiah *et al*. (1997) predicted, in relation to all possible combination of species an island can theoretically harbor, not only that small islands would harbor fewer species than large islands, but also that species composition of small islands when compared among other small islands would be more diverse than would be found in comparisons among the large islands, but also that the coefficient of variation of the similarities among sholas of similar sizes decreases with the size of the islands. Tests of these predictions have since been offered by studies from two relatively long-lived forest fragments, namely the sacred groves and the shola forest fragments of the central Western Ghats (Ganeshaiah *et al*. 1997; Tambat *et al*. 2001).

In the present study, we applied the predictions of Ganeshaiah *et al*. (1997) to the genetic status of fragmented populations in central Western Ghats using *Litsea floribunda* Gamble, belonging to the family Lauraceae. Our hypothesis was that, even from studies of a single widespread species, the genetic diversity (or collection of set of alleles at a locus) of small fragments would be more dissimilar among themselves than would be found among a set of large fragments. As co-adapted species are selected in an island, so too could co-adapted genes in a single species also be observed as being selected in an island. We will specifically be addressing the two predictions of Ganeshaiah *et al*. (1997): (a) whether the gene assemblages among a set of larger forest fragments are more similar to each other than among a set of small fragments? and (b) are the coefficient of variation for the similarity in the genetic status of population higher for smaller than the larger forest fragments? However, whereas Ganeshaiah *et al*. (1997) observed the evidence of from species assemblages, we looked at the evidence from the genomic DNA of a single widespread tree species.

METHODS

Study site

We tested the above predictions using the 'shola' forests that exist as isolated fragments in the upper reaches of the evergreen forests in Brahmagiri Wildlife Sanctuary (BWS; 11° 55' - 12° 8' N; 75° 44' - 76° 03' E) of the central Western Ghats, Karnataka State, India. The shola fragments are typical of the south Indian high altitude forests, which bear stunted vegetation and occur in the valleys and slopes of hills in discrete patches set amidst a grassland landscape found in the Western Ghats. These shola fragments are rich in Lauraceae members and are set as "islands" in a forest amidst a "sea" of grasses (**Fig. 1**). The sholas are "old" natural forest fragments which are suggested to have originated 35,000 years BP (Puri *et al*. 1989; Gupta 1989; Ganeshaiah *et al*. 1997). They provide a good model system for natural forest fragmentation to examine the issues related to long-term genetic consequences of the plant populations.

Study system

The study was carried out on *L. floribunda*, a middle storey tree. Trees flower annually during March-August and are known to be pollinated by insects. The fruits are berries and are generally animal dispersed. As *L. floribunda* was found in all the shola fragments (Saldanha 1984, 1996), at BWS, it formed an ideal study system to test our predictions.

Mapping the shola fragments

A detailed census of shola fragments of BWS were carried out to confirm the occurrence of *L. floribunda* tree species. Depending

on their occurrence, the shola fragments were classified into three size-classes, *viz*., small (<0.15 ha), medium (0.15 ha to 1 ha) and large (>1.0 ha). For genetic diversity studies, four small, four medium and three large shola fragments were selected. The latitude and longitude of identified sholas were recorded at several points along the perimeter for each size-class using a Global Positioning System (Garmin GPSmap 76S). The values were transferred onto a vector form using GIS software (MapInfo ver 5.0) and maps of their spatial distribution, shape, sizes and distances among shola fragments were arrived at (**Fig. 1**). The average distance that separates each shola fragment is about 1.6 km.

Sampling for genetic diversity study

Mature leaf samples of *L. floribunda* were randomly collected from eight individuals from each of the four small, four medium and three large shola fragments. The harvested leaves were wrapped in aluminum foil, frozen in liquid nitrogen and transferred to -70°C for further use. Genomic DNA, from the leaves of *L. floribunda*, was extracted using the CTAB method (Saghai-Maroof *et al*. 1984). The template genomic DNA was amplified by the polymerase chain reaction (PCR) in a programmable thermal controller (PTC-100™, MJ Research Inc.). Initially, 60 randomly amplified polymorphic DNA (RAPD) primers (www.operon.com) were screened, of which ten primers (OPG2, OPG3, OPG10, OPG11, OPG13, OPG14, OPG17, OPG19, OPW13 and OPX10) gave unambiguous banding patterns and hence were selected for further analysis. The PCR reaction mixture contained 25 µl of 50 mM Tris-HCL (pH 8.3), 250 µg/ml bovine serum albumin, 2% Ficoll, 1 mM Tartrazine, 2.5 mM $MgCl_2$, 2 mM dNTPs, 0.3 µM primer, 0.2 unit *Taq* polymerase (Bangalore Genei, India) and 50-75 ng of the template DNA. The PCR program used for amplification consisted of an initial denaturation at 94°C for 4 min, followed by 40 cycles at 94°C for 5 sec (denaturation), 35°C for 5 sec (annealing), 72°C for 2 min (extension). The final extension was carried out at 72°C for 8 min. Amplified PCR products were resolved on 1.5% agarose gels with 1X TBE buffer (pH 8.0) and were detected under UV light after ethidium bromide staining.

Scoring the gels

The presence or absence of RAPD bands were scored across the samples by following binary coding method (Wendel and Weeden 1989). Presence of a PCR amplified product was scored as '1' and its absence as '0'. Based on the absence or presence of amplified products, various genetic diversity parameters were estimated using statistical software tools such as POPGENE ver 1.32 and STATISTICA ver 4.5.

Data analysis

1. Mean correlation coefficient and coefficient of variation for the amplified product

The frequency of the PCR amplified products were computed over all the loci and a simple correlation analyses were performed among the various shola fragments within a given size-class (small, medium and large). Thus, for both the four small and four medium shola fragments, there are $4C_2$ combinations or pairs giving 6 correlation coefficients for each size class. Similarly, $3C_2$ combinations give 3 sets of correlation coefficients for the three large shola fragments. The mean coefficient of correlation and their respective coefficient of variance were further statistically derived for each size class (**Table 1**).

2. Mean similarity and frequency distribution of the similarity index

Based on the presence or absence of RAPD-PCR amplified products at a given loci, the similarity index (1-Squared Euclidean Distance) was calculated for all pairs of individuals across each size-class of the shola fragments. Among small and medium shola fragments, the mean was computed over $32C_2$ pairs giving 496 similarity indices, while that for among the large shola fragments, it was analyzed over $24C_2$ pairs giving 276 similarity indices. The mean similarity index variation over all pairs of individuals in a given size-class shola fragments were then compared using STATISTICA ver 4.5. Differences of mean similarity indices between shola size-classes were also statistically tested by the *t*-test. The frequency distribution of the similarity index for small, medium and large shola fragments were also tested for statistical significance by performing a *K-S* test (Siegel and Castellan 1988).

RESULT

The 10 short-listed RAPD primers gave a total of 146 unambiguous electromorphs. The probability levels at which the mean correlations were significantly different among the various size-classes of shola fragments is represented in **Table 1**. The mean correlation for the frequency of the PCR-amplified products was lower for the small (0.565 ± 0.026) compared to medium (0.626 ± 0.013; *t*-test, $p<0.06$) and the large shola fragments (0.636 ± 0.024; *t*-test, $p<0.13$). The range of correlation coefficients was wider for the small (0.48 to 0.67) compared to that for the large shola fragments (0.60 to 0.68). The coefficient of variation (CV) for the correlation among the small shola fragments was higher (0.046) than it was (0.037) for the large shola fragments (**Table 1**).

For the 32 individual trees in both the small and medium fragments, when analysed in pairs, there were 496 similarity indices, and for the 24 individual trees in the large fragments there were 276 similarity indices. The mean similarity index was least among the small shola fragments (0.657 ± 0.003) and highest among the large shola fragments (0.693 ± 0.004; *t*-test, $p<0.0001$; **Table 2**). The range in the similarity indices was wider for the smaller shola fragments (0.46 to 0.87) as compared to the larger shola fragments (0.53 to 0.85).

The frequency distribution of the similarity index between all pairs of individuals for each shola fragment within a given size-class was developed and compared using *K-S* test (Siegal and Castellan 1988). The frequency distribution of the similarity indices for the large shola fragment was skewed to the right compared to that for the small shola fragment, i.e., there was a higher representation of greater similarity indices in the large compared to the small shola fragments (**Fig. 2**). The differences in the distribution were statistically significant based on *K-S* test (Siegal and Castellan 1988).

The above analyses were repeated with amplification products that were polymorphic (at <95% criteria; data not shown). The resulting pattern of the similarity indices was

Table 1 Mean correlation of the frequency of RAPD-PCR amplified products (over all loci) for a given size-class of shola fragments for *Litsea floribunda* Gamble in the central Western Ghats, India.

Shola	N[a]	Range (r)	Mean[b] ± SEM[c]	CV[d]
Small	6	0.48 - 0.67	0.565 ± 0.026^{xy}	0.046
Medium	6	0.57 - 0.67	0.626 ± 0.013^{yz}	0.020
Large	3	0.60 - 0.68	0.636 ± 0.024^{xz}	0.037

[a]Sets of correlation coefficients over which the mean and standard deviation were computed
[b]Mean correlation
[c]Standard Error of the Mean
[d]Co-efficient of variation
t-test for differences in mean values between small(x), medium(y), and large(z) (xy; $p<0.06$, xz; $p<0.13$, yz; not significant) shola fragments

Table 2 Mean similarity index of RAPD-PCR amplified products (over all loci) among all individuals for a given size-class of shola fragments for *Litsea floribunda* Gamble in the central Western Ghats, India.

Shola	N[a]	Range (SI[b])	Mean[b] ± SEM[c]
Small	496	0.46 – 0.87	0.657 ± 0.003^{xy}
Medium	496	0.37 – 0.83	0.665 ± 0.003^{yz}
Large	276	0.53 – 0.85	0.693 ± 0.004^{xz}

[a]Pairs of individuals for which similarity indices was obtained
[b]Mean similarity Index
[c]Standard Error of the Mean
t-test for differences in mean values between small(x), medium(y), and large(z) (xy; $p<0.06$, xz and yz; $p<0.0001$) shola fragments

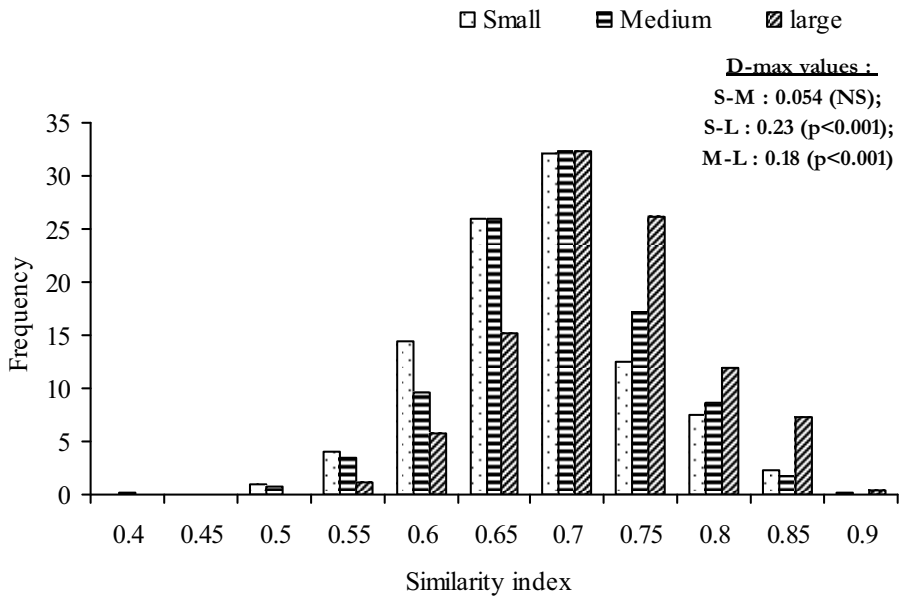

Fig. 2 Frequency distribution of similarity index of RAPD-PCR amplified products (over all loci) among all individuals from small (S), medium (M) and large (L) sized shola fragments for *Litsea floribunda* Gamble in the central Western Ghats, India.

similar to that reported above in that it suggested that the smaller shola fragments were more diverse among themselves than were a set of large shola fragments amongst themselves.

DISCUSSION

Island biogeography theory (MacArthur and Wilson 1967) has strong predictive power for the number of species on an island. This prediction is based on the assumption that the rate of immigration of species decreases and that of local extinction increases with increase in the number of species in the island. However, neither does this theory state the composition of the species in the islands nor does it say about the genetic structuring of the island populations that may be in diverse equilibrium states. Recently, Ganeshaiah *et al*. (1997) offered new insights into the species composition of islands with respect to their sizes, assuming a global set of "N" species that can randomly fill islands of different sizes. In the present study, we have applied the predictions of Ganeshaiah *et al*. (1997) to the genetic states of the population in the islands. We observed that as a set of co-adapted species are selected in an island, a set of co-adapted genes in a species could also be selected in that island.

The coefficient of variation for the mean correlation decreases with the size of the shola fragment (**Table 1**). Also, mean variance for all the amplified products for individuals from smaller shola fragments was higher when compared to the large and medium sized shola fragments. It suggests that the small shola fragments were at diverse equilibrium state (Ganeshaiah *et al*. 1997), which had assorted independently. The mean similarity for small shola fragments was significantly lower than the mean similarities for the medium and large shola fragments (**Table 2**). Similar studies on species assemblages in the high montane natural shola forests of Biligiri Rangan Swamy Temple Wildlife Sanctuary (BRTWS) along the edges of Western Ghats, India, Ganeshaiah *et al*. (1997) reported that small sholas showed higher coefficient of variation for the correlation coefficients for proportion of species shared in the medium and large shola forests. The species assemblage diversity was also tested using dung beetles in these BRTWS shola forests and only a small subset of species occurred in smaller dung pats (Ganeshaiah and Uma Shaanker 2003). The range of similarity index was wider for small and medium shola fragments as compared to the large shola fragments. The D-max value for the *K-S* test was also significant between small and large shola fragments for the frequency distribution (**Fig. 2**).

In other words, smaller shola fragments differ widely for the alleles shared among themselves as compared to the medium and large shola fragments. Larger shola fragments share most of the alleles as compared to the small or the medium shola fragments, which was evident from the frequency distribution of the similarity indices. Our results showed that certain alleles tend to persist in a given population suggesting that the populations in the small shola fragments may have evolved independently after they had colonized from a founder population over the years. Further, in the small populations with occasional inter-subpopulation migrations would preserve higher heterozygosity than the large intact populations (Boecklen 1986; Young and Boyle 2000). Vellend (2003) used 14 compiled data sets from the literature and showed a positive correlation between species and the genetic diversity across island areas. When the population size is larger, they tend to be panmictic, and hence the larger shola fragments were supposed to have higher similarity among themselves. In the larger shola fragments, there may be a greater probability of random exchange of alleles, while in the smaller shola fragments such complexes could have been conserved due to restricted gene exchange. In other words, larger shola fragments seem to converge towards a common equilibrium state showing higher similarity among themselves as compared to the smaller shola fragments. Our results also suggested that though individually the smaller shola fragments may not be diverse because of their small population size as compared to the large shola fragments, but collectively they add substantially to the spatial and structural heterogeneity of the ecosystem that might be important for its functional diversity. This diversity state is an important component of the ecosystem biodiversity because besides adding to the heterogeneity this state could have important functional properties. Ganeshaiah *et al*. (1997) also discussed that these patterns (small fragments more diverse than large) may not be true for all organisms. Large vertebrates that have wide home range, where the possibility of gene flow is common, cannot be expected to show such relations. On the other hand, these relations might not be uncommon for a number of lower organisms such as bacteria, fungi and lower vertebrates and invertebrates, which require small areas for their survival.

In conclusion, the study provides experimental evidence to test on the evolution of assortative equilibrium states in islands or natural fragments with respect to their genetic

states. The finding that a collection of smaller fragments may be more diverse in their gene assembly than a set of large fragments has important implications for the conservation of genetic diversity of populations in fragmented landscapes. Our study opens a broad scope of opportunities, both theoretical and empirical, for further exploring the correlates and causes of the species-genetic diversity relations with respect to natural forest fragmentation across many parts of the world.

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