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Impacts of habitat fragmentation on genetic diversity in a tropical forest butterfly on Borneo

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Abstract: Many areas of rain forest now exist as habitat fragments, and understanding the impacts of fragmentation is important for determining the viability of populations within forest remnants. We investigated impacts of forest fragmentation on genetic diversity in the butterfly *Mycalesis orseis* (Satyrinae) in Sabah (Malaysian Borneo). We investigated mtDNA diversity in 90 individuals from ten forest sites typical of the sizes of forest remnants that currently exist in the region. Nucleotide diversity declined with increasing isolation of remnants, but there was no effect of remnant size or population size, and haplotype diversity was similar among sites. Thus, approximately 50 y after forest fragmentation, few changes in genetic diversity were apparent and remnants apparently supported genetically viable populations of this butterfly. Many studies have shown that responses of species to habitat fragmentation usually follow a time delay, and so we developed a Monte Carlo simulation model to investigate changes in genetic diversity over time in small remnants. Model output indicated a substantial time delay (> 100 y) between fragmentation and genetic erosion, suggesting that, in the smallest study remnants, an increased risk of extinction from reduced genetic diversity is likely in the longer term.

Key words: forest remnants, genetic erosion, Lepidoptera, *Mycalesis orseis*

INTRODUCTION

Throughout the world, previously extensive tracts of natural habitat now exist as isolated fragments scattered across inhospitable landscapes (Groombridge & Jenkins 2000). This is evident in tropical regions where remaining forest patches support increasingly isolated populations of forest-dependent species (Brook *et al.* 2003). Tropical rain forests support the majority of global biodiversity and so understanding the impacts of habitat fragmentation in these areas is crucial for the conservation of biodiversity (Laurence & Bierregaard 1997, Turner 1996).

Populations within habitat fragments are expected to have lower genetic diversity than those in continuous habitats, due to restricted gene flow, genetic drift and increased inbreeding (Frankham *et al.* 2002). There is much debate about the importance of genetic factors in causing extinctions (Brook *et al.* 2002, Lande 1988, Speilman *et al.* 2004) but reduced genetic diversity is known to decrease the long-term persistence of populations in the wild (Saccheri *et al.* 1998). In addition, positive relationships between species diversity and allelic diversity support the importance of genetic factors in the preservation of biodiversity (Cleary *et al.* 2006). However, most studies investigating genetic consequences of fragmentation in tropical regions have focused on vertebrates (Brown *et al.* 2004) and data for invertebrates are lacking (Bickel *et al.* 2006), even though arthropods comprise the majority of biodiversity in terms of species richness (Groombridge & Jenkins 2000).

Previous studies have shown that the response of species richness to habitat change is not instantaneous, but usually occurs after a time delay (Diamond 1972, Tilman *et al.* 1994). Such delays vary from years to

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centuries depending on the taxon and the severity of fragmentation (Brooks & Balmford 1996, Brooks *et al.* 1999, Ferraz *et al.* 2003). Genetic changes following fragmentation also involve time delays (Ewers & Didham 2005, Frankham *et al.* 2002), but information is lacking on the likely time scale of such delays in the wild (Keyghobadi *et al.* 2005).

The Malaysian state of Sabah was originally almost entirely covered by rain forest, of which only 58% was remaining in 2001 (McMorrow & Talip 2001). Mechanization of timber extraction was developed in the 1950s leading to rapid conversion of forest to other land uses, and many existing forest remnants were probably isolated at this time (Benedick 2005). These remnants now exist as differently sized patches surrounded by large areas of oil palm (*Elaeis guineensis* Jacq.) plantation and other forms of agriculture. In this study, we investigated genetic diversity of the satyrine butterfly *Mycalesis orseis* Hewitson within these forest remnants. This species is known to be dependent on closed-canopy forest (Hamer *et al.* 2003) and does not occur in oil palm (Benedick 2005), and thus populations in forest remnants are likely to be genetically isolated. We tested the hypothesis that genetic diversity in forest remnants was related to population size, forest remnant size and degree of isolation. In order to investigate potential time delays between fragmentation and genetic erosion, we also developed a simulation model to determine whether or not small remnants are likely to support viable populations in the longer term.

METHODS

Insect material

Ninety adult *M. orseis* were sampled between November 2001 and August 2003 at 10 sites in Sabah comprising two widely separated control sites within a single large area (*c.* 1 million ha) of contiguous forest and eight sites within forest remnants varying in size from 120 ha to 120 000 ha. In order to sample butterflies, 20 fruit-baited traps were set up along a 2-km transect in each of the ten study sites. Each trapping session (two per site) lasted for 12 consecutive days and, across all sites, we trapped an average of 33 (range = 9–132) individual *M. orseis* per site (excluding recaptures). We analysed nine individuals per site; in order to have equal sample effort across sites for our molecular analyses, our sample size was limited by the number of individuals at sites with lowest abundance (five sites yielded only nine individuals). Study fragments were chosen to span the entire range of sizes of remaining forest remnants across Sabah and at the time of sampling, these were surrounded by an agricultural landscape comprising mainly oil palm. Isolation of each fragment, measured in terms of the minimum distance to contiguous forest using a regional map of Sabah, varied from 6 km to 69 km. Insect material was stored in 100% ethanol prior to analysis at the University of York (UK).

Molecular analysis

We investigated genetic diversity of mitochondrial (mt) DNA, a method which previously has been used successfully to examine fragmentation effects (Brown *et al.* 2004, DeChaine & Martin 2004). We chose this approach in preference to other molecular methods such as microsatellite markers, which are difficult to develop in Lepidoptera (Nève & Meglécz 2000), or the analysis of allozymes, which was not possible because access to low-temperature freezers for storage of insect material was not available. However, one disadvantage of this approach is that because mtDNA is maternally inherited, diversity estimates will be affected if males and females differ in their dispersal among sites (Frankham *et al.* 2002).

Total genomic DNA was extracted from thorax tissue using the DNeasy tissue kit (QIA), and then amplified by the polymerase chain reaction (PCR) prior to sequencing. The PCR primers 'George' (forward) 5′ATACCTCGACGTTATTCAGA 3′ and 'BtLYS' (reverse) 5′GTTTAAGAGACCAGTACTTG 3′ were used under standard reaction conditions (Monteiro & Pierce 2001). This yielded 796 bp of the cytochrome oxidase subunit II (COII) region of the mitochondrial genome. PCR products were purified using the QIAquick PCR purification kit, and sequenced in both directions using Applied Biosystems Big Dye terminators and visualized on an ABI 3110 automated sequencer (University of Oxford, UK).

Sequences were viewed and edited with Chromas version 1.45 software (http://www.technelysium.com.au/chromas.html) and visually checked. Contigs were assembled and edited with DNASTAR SeqMan software 4.03 (http://www.dnastar.com). Sequences were aligned using Bioedit Version 5.09 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), ClustalX (http://www-igbmc.u-strasbg.fr/BioInfo/ClustalX/Top.html), and by eye. Sequences were then imported into DnaSP version 3.99 (http://www.ub.es/dnasp/) to estimate haplotype (h) and nucleotide (π) diversity (Nei & Kumar 2000).

Statistical analysis

To determine the stability of our estimates of genetic diversity, we used bootstrapping methods (Colwell & Coddington 1994) to examine how the estimated number of haplotypes at each site changed with increasing sampling effort; each estimate was the mean of nine random selections from among the individuals sampled

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at each site, to remove sample order effects. We then used stepwise multiple regression to relate haplotype diversity and nucleotide diversity in each forest remnant to remnant area, remnant isolation and population size. Hierarchical levels of genetic differentiation within and among sites in four different size-classes (Table 2; continuous forest = sites 1 and 2, large remnants = sites 3 and 4, medium-sized remnants = sites 5 and 6, small remnants = sites 7–10) were next analysed with AMOVA (Excoffier *et al.* 1992) using Arlequin software (http://cmpg.unibe.ch/software/arlequin3/). Significance tests were based on 10 000 permutations of resampled data and pairwise F_{ST} values were estimated from the Tamura–Nei distance method (Tamura & Nei 1994). To test whether genetic differences between pairs of sites increased with increasing geographical separation of sites, Slatkin's pairwise linearized F_{ST} values (from the AMOVA analysis) were correlated with distance between sites, measured from a regional map of Sabah, using a Mantel test.

Modelling changes in genetic diversity following fragmentation

Our field study examined a period of approximately 50 y post-fragmentation, but genetic erosion may lag behind habitat changes. In order to examine long-term changes in genetic diversity following forest fragmentation, we developed a Monte Carlo simulation model to investigate loss of COII haplotypes over time in populations of different sizes. As is often the case for tropical species, few data exist on the ecology of *M. orseis*, making it difficult to parameterize some aspects of the models, and so data were taken from elsewhere. At each generation in the model, individuals were born, reproduced and died, and the population was completely isolated i.e. there was no immigration. The generation time of *M. orseis* is not known, and the model assumed non-overlapping generations; this may not be a valid assumption for this species but was unlikely to affect our results (Frankham 1995).

We investigated genetic diversity across a range of population sizes. We estimated the population density of *M. orseis* from field data using fruit-baited traps to sample butterflies at study sites (see above; Benedick *et al.* 2006). We estimate that our 20 fruit-baited traps set up along a 2-km transect sampled an area of approximately 15.7 ha at each site (traps were 100 m apart and we assumed each trap operated over a circular area of radius 50 m; Hamer & Hill 2000). It is likely that *M. orseis* breeds continuously in Sabah given the lack of marked wet and dry seasons in the region, and a previous study indicates that tropical butterflies can develop through six generations y^{-1} (Azerefegne *et al.* 2001). Over a 24-d period, we trapped between 9 and 132 individual *M. orseis* per site (Table 2). Thus, we estimate between 23 and 335 individuals of *M. orseis* per generation per transect (i.e. over a 60-d period). Assuming each transect sampled an area of 15.7 ha, this results in estimates of population density ranging from 1.5 (sites 6, 7, 9 and 10) to 21 (site 8) individuals ha^{-1}, and estimated population sizes in the four smallest remnants < 1000 ha of 1086 individuals (site 7), 13 566 individuals (site 8), 752 individuals (site 9) and 180 individuals (site 10).

Our estimates of population sizes in the smallest remnants were based on many assumptions for which supporting data were non-existent, or were for other species. Given that population densities varied considerably among these small fragments, we investigated changes in genetic diversity across a range of population sizes (from 100 to 5000 individuals). For each simulation, the model was seeded with individuals according to the population size. Each subsequent generation was produced by randomly sampling females from the previous generation. We assumed that the number of offspring produced by each female was drawn from a normal distribution with a mean of 30 and variance of 10. We assumed a 50:50 sex ratio and offspring were subsequently assigned a probability of mortality (distributed equally among individuals) based on the size of the population. In general, modelled population sizes over time varied by no more than 10% of the initial population size.

Mycalesis orseis from the two 'control' sites in continuous forest were characterized by seven COII haplotypes in total, and so models were seeded with each individual assigned one of seven haplotype states, drawn at random from a uniform distribution, to represent the starting levels of genetic variation in the population prior to fragmentation. At each generation, genes could mutate according to the 'infinite alleles model' such that each new mutation produced a unique haplotype. The mutation rate was calculated following Brower (1994) who estimated 2.3% pairwise sequence divergence in a sequence of 950 bp of mtDNA per million years in arthropods. Thus we estimated 5×10^{-6} mutations per 796 bp length of sequence per generation in *M. orseis*. We ran models for 300 generations, assuming that the butterfly develops through six generations y^{-1} (i.e. 60-d generation time; Azerefegne *et al.* 2001) and that study populations have been isolated for about 50 y (Benedick 2005, McMorrow & Talip 2001). Haplotype diversity and the number of haplotypes in the population were calculated for each population size for all individuals in the populations as well as for a random sample of nine individuals (the number of *M. orseis* sampled at each locality in this study). Simulations were replicated 200 times for each population size to allow confidence limits to be estimated. In order to determine how genetic diversity

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may vary over time in study populations, we also ran models for 100 y (≈ 600 generations) and 200 y (≈ 1200 generations).

Clearly some of our parameter estimates were more reliable than others, and so we tested the sensitivity of the model to parameter estimates by varying each of the four parameters (mean offspring per female, variance in offspring number, sex ratio and mutation rate) between −50% and +50% (in intervals of 5%) of our estimate, while keeping the other parameter values constant. For each parameter, 30 replicates were run, enabling the calculation of correlation coefficients between the parameter and haplotype diversity, and between the parameter and the number of haplotypes.

RESULTS

Molecular genetic data

We identified 12 polymorphic positions along the 796 bp sequence of the COII gene, which revealed 18 unique haplotypes in 90 *M. orseis* (Table 1) from 10 forest locations (Figure 1). Estimated number of haplotypes increased with the number of individuals sampled and was reasonably stable as final sampling effort was approached at each site (Figure 2). Mean values for haplotype (h) and nucleotide (π) diversity were 0.756 (SD = 0.13) and 0.0025 (SD = 0.0012), respectively, across all samples (Table 2). Relationships between the 18 haplotypes are shown in Figure 3, and Table 3 shows the relative frequencies of the 18 haplotypes at each study site. There was no relationship between haplotype diversity (h) in forest remnants and either remnant size, remnant isolation or butterfly population size (multiple regression; $P > 0.1$). However, nucleotide diversity (π) decreased significantly with increasing remnant isolation (Figure 4; $F_{1,6} = 11.0, P = 0.02, R^2 = 0.65$). Isolation accounted for 65% of the variation in nucleotide diversity, and there was no relationship with remnant area or population size ($P > 0.9$ in both cases). Haplotype and nucleotide diversity in the largest forest remnants were no different from those in intact forest ($t_2 = 0.2$, $P = 0.8$ and $t_2 = 0.6$, $P = 0.6$ respectively).

We used hierarchical AMOVA to examine the partitioning of molecular variance within and among forest fragments of different sizes (continuous, large, medium, small). This analysis revealed that most variance (> 80%) was within sites (F_{ST} = 0.12, df = 80, N = 90, $P < 0.002$), with a further 15% distributed among sites within fragment size classes (F_{ST} = 0.15, df = 6, $P < 0.004$), but no significant variance distributed among the different fragment size classes ($P > 0.7$). There was no significant correlation between geographical distance and genetic distance (pairwise F_{ST} values) between sites (Table 4; Mantel test, r_m = 0.14, P = 0.56) indicating an absence of any isolation by distance for F_{ST} values.

Table 1. Genetic diversity was examined in *Mycalesis orseis* from ten forest sites in Sabah (Malaysian Borneo). Data show nucleotide variability among the 18 haplotypes of the COII gene obtained from 90 individuals of *M. orseis* from the 10 study sites. Number of individuals with each haplotype is given in parentheses. Dots indicate where the same nucleotide occurs in the sequence. Nucleotide position numbers indicate the positions of polymorphic sites along the 796-base pair sequence (which corresponds to positions 2774-3808 in the *Drosophila yacuba* mtDNA sequence; Monteiro & Pierce 2001).

	Nucleotide position												
Haplotype	34	135	217	363	381	438	549	558	600	655	693	735	768
1 (1)	A	G	T	A	G	T	C	A	A	A	G	C	G
2 (1)	.	A	.	.	.	.	T	.	.	.	.	.	.
3 (1)	.	.	.	.	.	.	T	G	.	.	.	.	.
4 (1)	.	.	.	.	.	.	T	.	.	G	.	.	.
5 (29)	.	.	.	.	.	.	T	.	.	.	.	.	.
6 (1)	.	.	.	G	.	.	T	.	.	.	A	.	.
7 (15)	.	.	.	.	.	.	T	.	.	.	A	.	.
8 (8)	.	.	.	.	.	.	T	.	.	.	.	.	A
9 (14)	.	.	.	.	.	.	T	.	.	.	A	.	A
10 (1)	G	.	.	.	.	C	T	.	G	.	A	.	.
11 (1)	.	.	.	.	.	.	T	.	G	.	A	.	A
12 (1)	.	.	.	.	.	.	T	.	G	.	A	T	A
13 (1)	G	A	.	.	.	.	T	.	G	.	A	T	A
14 (1)	G	.	.	.	A	.	T	.	G	.	A	T	A
15 (7)	G	.	.	.	.	.	T	.	G	.	A	T	A
16 (1)	G	.	C	.	.	.	T	.	G	.	A	T	A
17 (4)	G	.	.	.	.	C	T	.	G	.	A	T	A
18 (2)	G	.	.	.	.	.	T	.	G	.	.	T	A

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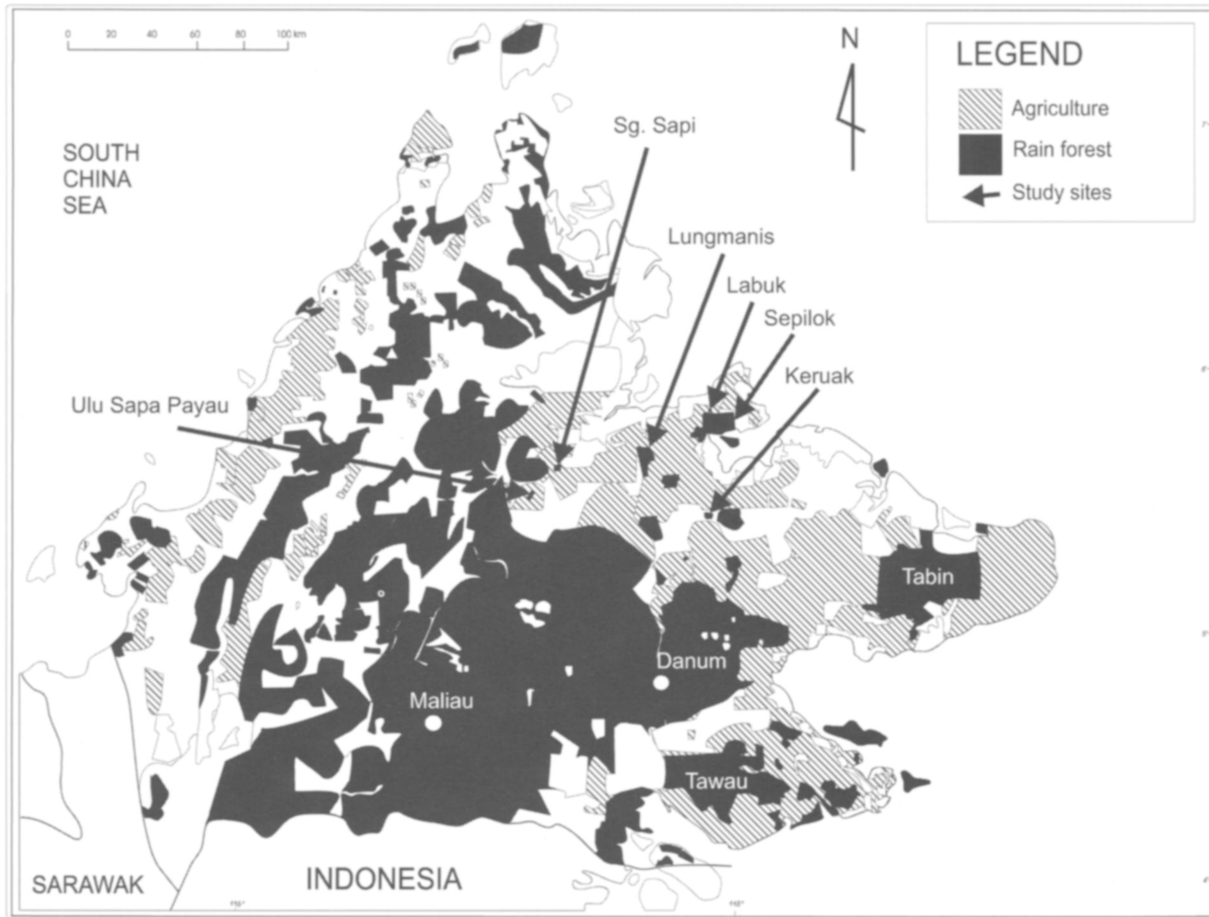

Figure 1. Map of Sabah (Malaysian Borneo) showing 10 sampling sites for *Mycalesis orseis*. The species was sampled in eight forest remnants and two 'control' sites in continuous forest. The forest remnants are surrounded by plantations of oil palm.

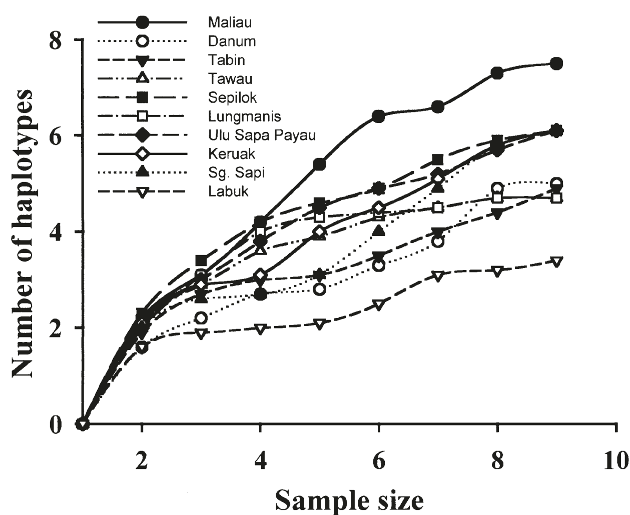

Figure 2. Genetic diversity was examined in *Mycalesis orseis* in rain-forest patches in Sabah, Borneo. Nine individuals of *M. orseis* were sampled from each site. The graph shows haplotype accumulation curves which illustrate changes in the estimated number of haplotypes with increasing sampling effort of *M. orseis*. Forest sites are listed in order of decreasing size.

Modelling output

We carried out sensitivity analyses to determine how variation in mutation rate, mean number of offspring per female (as well as variance in this value), and the sex ratio of offspring affected model output (Table 5). These analyses showed that within the range of values tested, haplotype diversity was sensitive to the mean number of offspring produced and to deviations from a 50:50 sex ratio, and haplotype number was sensitive to all parameter values. We would expect the model to be sensitive to changes in the sex ratio because mtDNA is maternally inherited and so altering the sex ratio to increase the male bias of offspring has the same consequence as reducing the overall size of the population, and vice versa. However, field data for *M. orseis* did not show a significant deviation from a 50:50 sex ratio ($\chi^2 = 3.31$, $P > 0.08$, $N = 322$ individuals) indicating that our parameter value of a 50:50 sex ratio was appropriate.

Predicted genetic diversity declined with decreasing population size and also declined over time (Figure 5).

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Table 2. Haplotype (h) and nucleotide (π) diversity indices (mean ± SD) for ten study sites. Remnant size and isolation of remnants from nearest contiguous forest are given. 'Abundance' = number of *Mycalesis orseis* sampled in fruit-baited traps over a 24-d period (excluding re-captures). Sites are listed in order of decreasing size: 1 = Maliau, 2 = Danum Valley, 3 = Tabin, 4 = Tawau, 5 = Sepilok, 6 = Lungmanis, 7 = Ulu Sapa Payau, 8 = Keruak, 9 = Sungai Sapi, 10 = Labuk. See Figure 1 for locations of sites.

Site	Area (ha)	Isolation (km)	Abundance	Number of haplotypes	h	π
1	*c.* 1 000 000	–	9	6	0.889 ± 0.091	0.0036 ± 0.0005
2	*c.* 1 000 000	–	17	4	0.583 ± 0.183	0.0010 ± 0.0003
3	122539	56	40	4	0.583 ± 0.183	0.0017 ± 0.0007
4	91587	44	71	4	0.806 ± 0.089	0.0013 ± 0.0002
5	4294	63	21	5	0.861 ± 0.087	0.0022 ± 0.0007
6	3390	38	9	4	0.806 ± 0.089	0.0034 ± 0.0007
7	720	6	9	5	0.861 ± 0.087	0.0038 ± 0.0008
8	640	28	132	5	0.806 ± 0.120	0.0027 ± 0.0007
9	500	25	9	5	0.806 ± 0.120	0.0043 ± 0.0007
10	120	69	9	3	0.556 ± 0.165	0.0009 ± 0.0003

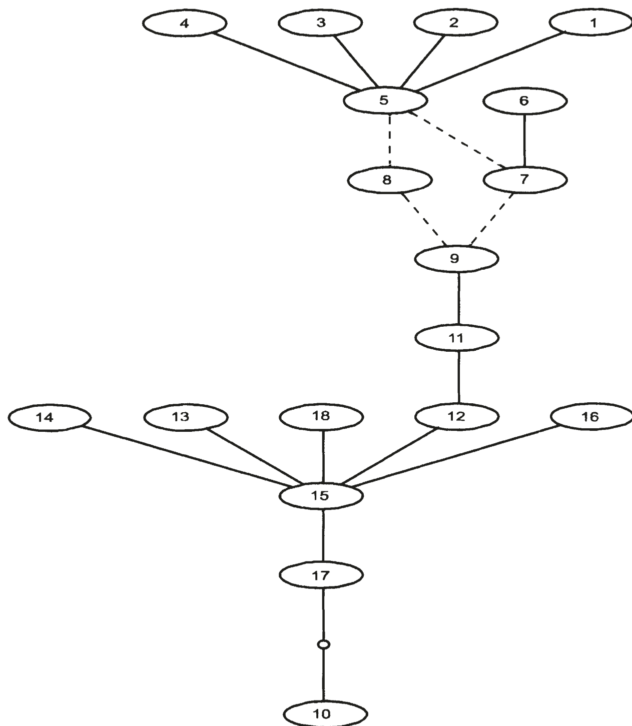

Figure 3. Haplotype network of *Mycalesis orseis* inferred using the criterion of parsimony (generated using TCS ALPHA 1.01). Haplotype number is shown within ellipses and dotted lines indicate where ambiguities are present. Each solid line represents a single mutation, and the small circle indicates a node in the network that was absent in the samples.

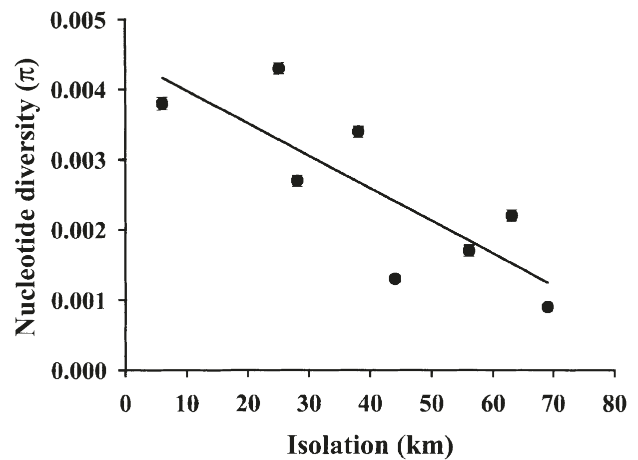

Figure 4. Nucleotide diversity (mean ± 1 SE) for *Mycalesis orseis* in rainforest remnants in Sabah, Borneo, in relation to isolation. The linear regression line is shown (regression, $F_{1,6} = 11.0$, P = 0.02, $R^2 = 0.65$) and is described by the equation: π = a – b(isolation), where a ± SE = 0.00445 ± 0.00064, and b ± SE = 0.000046 ± 0.000014. See Table 2 for information on site isolation.

Fifty years after fragmentation, genetic diversity reached an asymptote at fairly low population sizes, and little further increase in either haplotype number or diversity was simulated in populations greater than approximately 3000 individuals (Figure 5a, c). However, population sizes < 2000 (corresponding with three of the four smallest remnants in our study) were predicted to have substantially reduced diversity 50 y post-fragmentation. Simulated genetic diversity also declined over time such that even relatively large populations (3000 individuals) showed an approximate halving of diversity 200 y post fragmentation. Across the range of population sizes studied, there was a relatively long time delay between fragmentation and substantial genetic erosion. Figure 5b shows that predicted changes in haplotype diversity were similar regardless of whether the entire population was analysed, or just a sample of nine individuals from each population, although measures of haplotype number were more sensitive to sample sizes (Figure 5d).

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Table 3. Relative frequencies of 18 haplotypes of *Mycalesis orseis* at ten study sites. Sites are listed in order of decreasing size and numbering follows Table 2.

	Site									
Haplotype	1	2	3	4	5	6	7	8	9	10
1	–	–	–	–	–	–	0.11	–	–	–
2	–	–	–	–	–	–	–	–	–	0.11
3	–	–	–	–	–	–	–	–	0.11	–
4	–	–	–	–	–	–	–	0.11	–	–
5	0.33	0.11	0.67	0.33	0.22	0.33	0.33	0.44	0.22	0.22
6	–	–	–	–	–	–	–	0.11	–	–
7	0.11	0.11	–	0.33	0.11	0.33	–	–	–	0.67
8	–	0.11	0.11	0.11	0.33	–	0.22	–	–	–
9	0.11	0.67	0.11	0.22	0.22	–	–	0.22	–	–
10	–	–	–	–	–	–	–	–	0.11	–
11	–	–	–	–	–	–	–	–	0.11	–
12	0.11	–	–	–	–	–	–	–	–	–
13	–	–	–	–	–	–	0.11	–	–	–
14	–	–	–	–	–	0.11	–	–	–	–
15	0.22	–	–	–	0.11	0.22	0.22	–	–	–
16	0.11	–	–	–	–	–	–	–	–	–
17	–	–	–	–	–	–	–	–	0.44	–
18	–	–	0.11	–	–	–	–	0.11	–	–

DISCUSSION

Impacts of habitat fragmentation on butterfly genetic diversity

There was some evidence of a reduction in genetic diversity of *M. orseis* following forest fragmentation but this was restricted to a reduction in nucleotide diversity with increasing isolation of remnants (Figure 4). There was no effect of isolation on haplotype diversity and no relationship between any measures of genetic diversity and forest fragment size or estimated butterfly population size. Visual inspection of Table 2 also reveals little consistent pattern between butterfly abundance and genetic diversity in the contiguous forest sites. We conclude therefore that overall changes in genetic diversity following forest fragmentation were slight. This was probably due to the relatively large size of fragments in this study and, in support of this notion, previous studies showing reduced genetic diversity of butterflies in temperate regions following habitat fragmentation (Berwaerts *et al.* 1998, Saccheri *et al.* 1998, Williams *et al.* 2003) have generally involved much smaller fragments than those currently occurring in Sabah. For example, Saccheri *et al.* (1998) studied temperate habitat fragments < 3 ha, which would have supported substantially smaller population sizes than those considered here. Previous temperate studies have also shown an effect of habitat patch isolation on genetic diversity, in the absence of any patch area effects, supporting the findings in this study (Krauss *et al.* 2004).

In this study, there was some evidence of genetic erosion in more isolated fragments. This relationship could have arisen from dispersal of individuals between less-isolated sites (Fauvelot *et al.* 2006), but any such movements were unlikely in this study because *M. orseis* apparently is dependent on closed-canopy forest and does not occur within agriculture (Benedick *et al.*, 2006). Most

Table 4. Pairwise FST-values of *Mycalesis orseis* estimated from Tamura-Nei distances (below diagonal) and linear distances (km, above diagonal) between patches of lowland rain forest in Sabah, Borneo. Sites are listed in order of decreasing size and numbering of sites follows Table 2.

Site	1	2	3	4	5	6	7	8	9	10
1		100	213	131	178	134	88	138	106	166
2	0.17		113	78	97	78	82	53	88	103
3	0.16	0.34		116	116	141	181	103	172	125
4	0.16	0.14	0.08		166	150	156	119	159	166
5	0.04	0.02	0.03	0.01		38	91	38	78	10
6	0.08	0.17	0.11	0.07	0.03		56	38	41	34
7	0.06	0.17	0.01	0.10	0.03	0.04		81	20	84
8	0.07	0.17	0.07	0.04	0.04	0.01	0.02		69	44
9	0.02	0.34	0.30	0.33	0.22	0.06	0.08	0.23		72
10	0.20	0.42	0.25	0.04	0.25	0.14	0.23	0.09	0.40	

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Table 5. The sensitivity of the simulation model was examined in relation to changes in the values of the four parameters. Each parameter was varied and tested separately. For each parameter, values between − 50% and + 50% of the estimated value were incorporated into the simulations (in intervals of 5%). Thirty replicates were carried out for each parameter interval. Correlation coefficients of haplotype diversity and number of haplotypes with each of four tested variables are shown in the table; non-significant correlations indicated that the model output was relatively insensitive to the exact parameter values used in simulations.

Variable	Correlation coefficient	
	Haplotype diversity	Number of haplotypes
Mutation rate	0.043(NS)	0.141***
Mean offspring per female	0.079*	0.126***
Variance in offspring number	−0.053(NS)	−0.126***
Male bias in offspring	−0.418***	−0.588***

remaining continuous forest is in the interior of Sabah and the most isolated areas are generally near the coast (Figure 1). It is very likely that these more accessible coastal areas were the first to be logged following the development of mechanized timber extraction in the 1950s. Therefore the most isolated sites in this study have probably been isolated for the longest time, and thus our estimates of the effects of current isolation may be confounded by time since logging. The only previous study to investigate genetic erosion in tropical invertebrates in forest fragments was also carried out in Sabah (Bickel *et al.* 2006). In that study, Bickel *et al.* (2006) found a more marked reduction in genetic diversity in one species of leaf litter ant than was observed in our study. Bickell *et al.* (2006) sampled only a few sites, but they included the most isolated sites sampled in this study. This raises the possibility that isolation of fragments

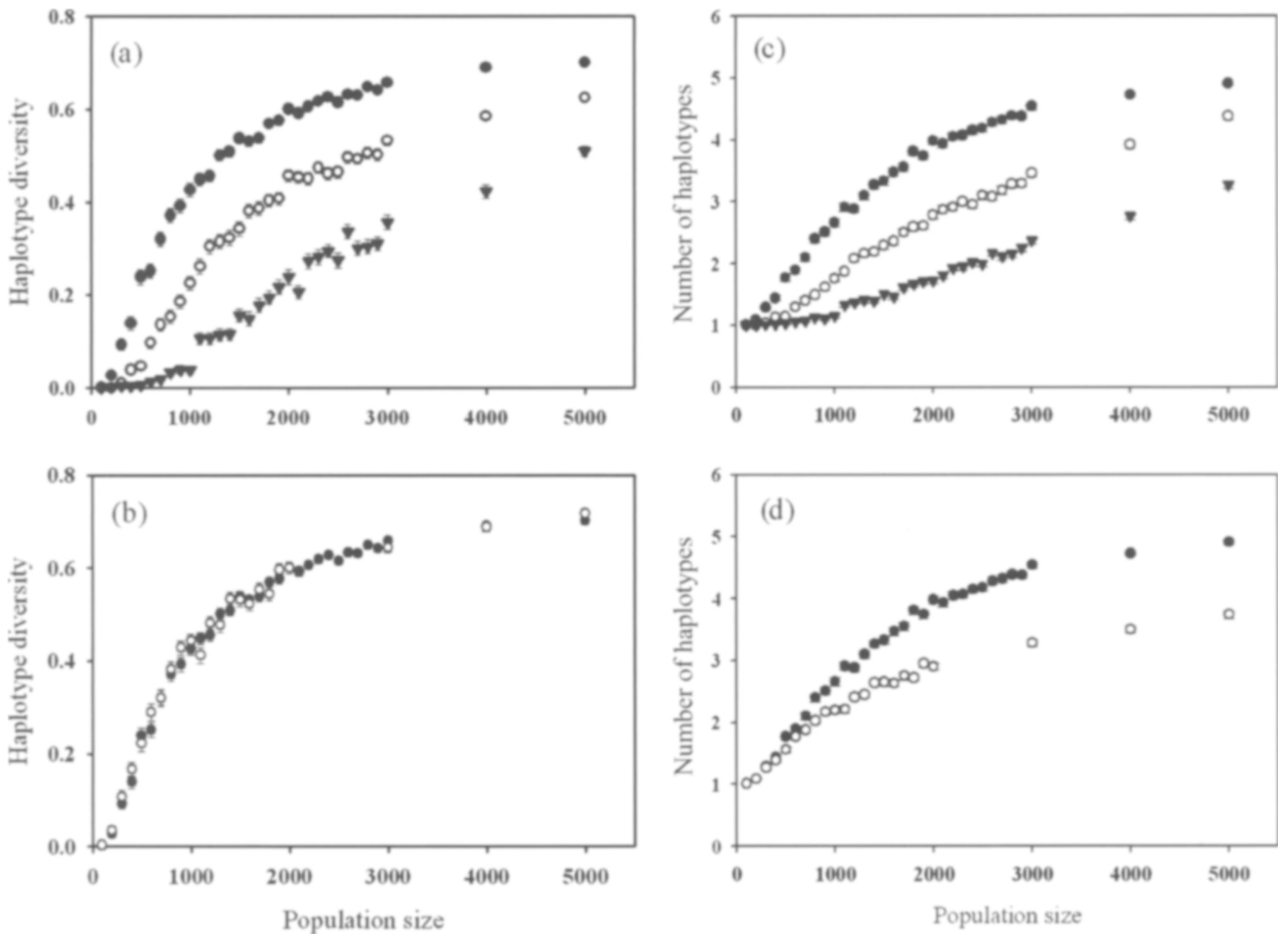

Figure 5. The time periods over which loss of genetic diversity might occur in *Mycalesis orseis* following forest fragmentation were simulated in small populations of different sizes. Panels show simulated changes in haplotype diversity (a, b), and number of haplotypes (c, d), in relation to population size. The top two panels (a, c) show changes in genetic diversity over time in the entire population, and the bottom two panels (b, d) show the effects of sampling just nine individuals. Panels a and c: haplotype diversity and number after 50 y (solid circles), 100 y (hollow circles) and 200 y (solid triangles). Panels b and d: haplotype diversity and number in the entire population (solid circles) and for a subsample of nine individuals (hollow symbols) after 50 y. Mean and SE are plotted, based on 200 runs of the model. Observed haplotype diversity and number of haplotypes for the smallest study fragment (120 ha) were 0.556 and 3 haplotypes, respectively.

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(either in space or time) is more important than size per se, although taxa may differ in terms of their response to fragmentation.

In this study, we focused on mtDNA diversity, but there is a wide range of other molecular genetic tools available for investigating genetic consequences of habitat fragmentation (Loxdale & Lushai 1998, Sunnucks 2000). Some studies have shown that different molecular markers produce qualitatively similar findings (Hoole *et al.* 1999, Fauvelot *et al.* 2006, but see Vandewoestijne & Baguette 2002) but examining a variety of different genetic markers may be a more reliable approach in the future (Haig 1998), especially since some markers may not be selectively neutral (Goulson 1993, Hurst & Jiggins 2005, Watt 1992). It is possible that the lack of any strong effects of fragmentation on genetic diversity in this study was because the mtDNA markers we used were not sufficiently sensitive. However, our techniques were sensitive enough to detect significant effects of remnant isolation on nucleotide diversity, as well as detecting genetic structuring among populations, suggesting that our methods would have detected any effects of remnant size if they had been present.

Changes in genetic diversity over time

Model output predicted that haplotype number and diversity in isolated forest fragments should decrease over time and with decreasing population size, as expected. Fifty years post-fragmentation, there was little predicted relationship between fragment size and genetic diversity above population sizes of approximately 3000 individuals. Thus, the model predicted that all forest fragments, with the possible exception of some of the smallest in our study, should currently support genetically viable populations. This prediction was in broad agreement with field data; for example, maximum predicted haplotype diversity after 50 y in the largest populations (mean = 0.70; Figure 5a) was similar to overall haplotype diversity observed across sampled populations (mean = 0.756). However, genetic erosion was simulated in the model more rapidly than we observed in the field. We estimated that three of our remnants with the smallest populations (sites 7, 9 and 10) had a mean population size of approximately 700 individuals which, according to our model, would be expected to have a mean haplotype diversity of 0.321, and a mean of 2.1 haplotypes, approximately half that observed (observed diversity = 0.741, mean number of haplotypes = 4.3; Table 2). Nonetheless, given that population sizes of study fragments are based on parameter estimates where supporting field data are lacking (see below), we are encouraged that the simulated diversity values fall within the likely range of population sizes of the smallest fragments ($\sim$ 100–2000 individuals). Thus, our model output provides good support for the presence of a time delay between fragmentation and genetic erosion, but precise estimation of the length of the delay is not possible without new empirical data for inclusion in the model.

There are several possible reasons why the model predicted that genetic erosion would occur slightly more quickly than observed. First, *M. orseis* may develop through fewer than six generations per year, which would result in slower genetic erosion than predicted. Second, our model assumes no gene flow among populations at any time, yet formation of forest fragments may not have been instantaneous but may have occurred gradually, allowing gene flow to continue during the early period of fragment formation. In addition, our population size estimates are based on butterfly captures in fruit-baited traps, but relationships between trapping frequency and population size have not been quantified; for example, if we over-estimated the area over which traps sample, then our estimates of population size will be conservative.

It is not clear exactly what degree of genetic erosion correlates with increased extinction risk, but previous work on butterflies indicated that a decline in diversity by approximately two-thirds resulted in extinction of local populations (extinction was observed only in populations with fewer than 2.5 heterozygous loci compared with a maximum of 7 heterozygous loci in surviving populations; Saccheri *et al.* 1998). An equivalent reduction in diversity in our model suggests that few populations ≤ 2000 individuals will be viable in the longer term. Importantly, the model predicts that although populations in the smallest fragments currently appear genetically viable, their diversity will in time decrease to well below two-thirds of the initial value pre-isolation. Thus small fragments may not be viable in the longer term.

We have assumed that population size is directly related to fragment area, but there is evidence that herbivore abundance may increase following fragmentation due to loss of predators (Terborgh *et al.* 2001). In this study, the population density of *M. orseis* did vary across sites, but there was no clear pattern in relation to fragment size, and small remnants supported both the highest and lowest observed population densities (Table 2). In addition, one of the control sites in contiguous forest apparently supported low butterfly density, but had high levels of genetic diversity suggesting that population size rather than local density may be more important in relation to genetic erosion. The sizes of fragments that currently exist in Sabah are unlikely to be sufficiently small to exclude all vertebrate predators of butterflies, and so greatly increased butterfly abundance in small fragments is unlikely. In addition, small fragments are likely to suffer increased habitat disturbance (Benedick 2005) and so interactions between genetic erosion and habitat disturbance are likely to be the most important

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factors increasing the probability of local extinction of species in small fragments in the long term.

It is clear that not all species are equally vulnerable to extinction (Koh *et al.* 2004), and species are also likely to differ in their sensitivity to genetic erosion, depending on their population genetic history, population size within fragments, and their dispersal ability. Previous studies have shown that *M. orseis* is not adversely affected by commercial selective logging (Hamer *et al.* 2003) and from nearly 90 species sampled in this study, *M. orseis* was one of only five species that occurred in all study locations. This suggests that *M. orseis* may be relatively insensitive to fragmentation compared with congeneric endemic species which were never sampled in small fragments. Medium-sized satyrines, similar to *M. orseis,* were also shown to persist in forest fragments in other studies (Shahabuddin & Terborgh 1999). The local populations of sensitive endemic species may disappear from small fragments due to population dynamic consequences of isolation (for instance, increased demographic stochasticity) or due to lack of suitable habitat, long before they suffer any long-term changes in genetic diversity.

Conservation implications

Our results suggest that forest fragmentation in Sabah dating from the 1950s has not resulted in a large reduction in genetic diversity in our study species, and that populations in even the smallest forest fragments currently are genetically viable. Sensibly, most conservation effort in Sabah has been focused on protecting large areas of undisturbed forest but this study supports the suggestion that small fragments also have conservation value and are worthy of protection (Benedick *et al.* 2006). However, in the absence of gene flow, populations in the smallest fragments probably are unlikely to be viable in the longer term. Our model indicates that the long time scales involved (decades to centuries) appear sufficient for conservation management to be implemented, such as the development of forest corridors to increase chances of gene flow among sites. Our results showed that more isolated remnants had reduced genetic diversity, and the continued expansion of agricultural land and urbanization could greatly reduce the size and/or increase the isolation of fragments and thus reduce the genetic viability of populations within them. Small fragments are also much more prone to habitat disturbance (e.g. fire, illegal logging), and so increased protection will be necessary to prevent their further degradation or complete destruction (Curran *et al.* 2004, DeFreis *et al.* 2005); in the immediate term, this is probably a more important consideration than any erosion of genetic diversity.

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