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Effects of habitat fragmentation on the genetic diversity and differentiation of *Dendrolimus punctatus* (Lepidoptera: Lasiocampidae) in Thousand Island Lake, China, based on mitochondrial COI gene sequences

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Abstract

Thousand Island Lake (TIL) is a typical fragmented landscape and an ideal model to study ecological effects of fragmentation. Partial fragments of the mitochondrial cytochrome oxidase subunit I gene of 23 island populations of *Dendrolimus punctatus* in TIL were sequenced, 141 haplotypes being identified. The number of haplotypes increased significantly with the increase in island area and shape index, whereas no significant correlation was detected between three island attributes (area, shape and isolation) and haplotype diversity. However, the correlation with number of haplotypes was no longer significant when the 'outlier' island JSD (the largest island) was not included. Additionally, we found no significant relationship between geographic distance and genetic distance. Geographic isolation did not obstruct the gene flow among *D. punctatus* populations, which might be because of the high dispersal capacity of this pine moth. Fragmentation resulted in the conversion of large and continuous habitats into isolated, small and insular patches, which was the primary effect on the genetic diversity of *D. punctatus* in TIL. The conclusion to emphasize from our research is that habitat fragmentation reduced the biological genetic diversity to some extent, further demonstrating the importance of habitat continuity in biodiversity protection.

Keywords: *Dendrolimus punctatus*, habitat fragmentation, genetic diversity, genetic differentiation, COI

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Introduction

Landscape fragmentation refers to the separation and division of a large and continuous landscape into smaller, isolated landscape fragments (Ranta *et al.*, 1998; Franklin *et al.*, 2002), which can be induced by human (Fahrig, 2003) or non-human factors (Bukey, 1995; Fagan, 2002; Leisnham & Jamieson, 2002; Watson, 2002). The negative effects of this process are

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decreases in overall habitat availability and quality and changes in the spatial configuration of habitats (Fahrig, 2003; Henle *et al.*, 2004). Both theoretical and empirical studies show that habitat fragmentation can erode neutral and adaptive genetic diversity of populations because of decreases in effective population size and inter-population connectivity (Johansson *et al.*, 2004; Marianna *et al.*, 2009; Wang *et al.*, 2011; Bao, 2013; Goossens *et al.*, 2016). Genetic diversity is required for evolution to occur, indicated by the expected relationship between heterozygosity and population fitness (Reed & Frankham, 2003). Therefore, landscape fragmentation threatens local, regional, national and ultimately global biodiversity (Tilman *et al.*, 1994; Dobson *et al.*, 1997), and as a consequence, habitat fragmentation has become a central concern in biological conservation.

Thousand Island Lake (hereafter TIL) is an artificial lake created by the construction of the Xin-An Jiang dam in 1958 and is located in the west of Hangzhou, Zhejiang, China. TIL, as an anthropogenic fragmented island landscape, is an ideal model to investigate ecological effects of fragmentation because of the well-delineated boundaries, inhospitable surrounding matrix, relatively homogeneous habitats and consistent isolation time for different island habitats (Yu & Mcgeoch, 2012; Bao, 2013; Jia *et al.*, 2016).

Many studies indicate that reductions in habitat area lead to reductions in population size and colonization rates, which increase the risk of species extinction (Bowers & Matter, 1997; Bender *et al.*, 1998; Hanski, 1998; Crooks *et al.*, 2001; Hames *et al.*, 2001; Schoereder *et al.*, 2004). Many studies also demonstrate that complex fragments have more frequent colonization than simple patches (Game, 1980; Collinge, 1996; Hamazaki, 1996; Bevers & Flather, 1999; Collinge & Palmer, 2002; Cumming, 2002). Additionally, genetic differentiation among invertebrate populations is clearly related to fragment isolation in many studies (Van Dongen *et al.*, 1998; Schmitt & Seitz, 2002; Krauss *et al.*, 2004), although not in all (Ramirez & Haakonsen, 1999; Wood & Pullin, 2002; Marianna *et al.*, 2009; Bao, 2013). In this study, we focus on *Dendrolimus punctatus* (Lepidoptera: Lasiocampidae), which is a moth that feeds on pine trees, and is a common invertebrate species in the local community. Because the species has approximately 2–3 generations per year (Fei *et al.*, 2014), approximately 170 generations had passed for the *D. punctatus* populations since potential isolation. Consequently, we suspect that the accumulation of genetic variation could be substantial and can allow for the testing of the following hypotheses: (1) habitat fragmentation will affect the genetic diversity and genetic differentiation among different populations of *D. punctatus* in TIL; (2) smaller island area and oversimplified island shape will decrease the genetic diversity of *D. punctatus* populations in TIL.

Materials and methods

Study area and population sampling

Our study was conducted on TIL (29°22″–29°50″N, 118° 34″–119°15″E), where we selected 23 islands (fig. 1) that were completely isolated by water. These islands were selected because they (1) represented a range of areas and degree of isolation, (2) had as little human disturbance as possible and (3) contained populations of *D. punctatus*. Attributes of the selected islands, including island area (at 105 m a.s.l.), isolation (distance to nearest island) and island shape index (SI) measured by GIS, are listed in table 1. Island SI is a measure of island shape complexity, which was calculated as: $SI = P/[2 \times (\pi \times A)^{0.5}]$ (Laurance & Yensen, 1991; Hoffmeister *et al.*, 2005; Ewers & Didham, 2007), where P is the island perimeter and A is the island area. The SI measures deviations from circularity, with a circle having an SI value of 1 and increasingly more complex shapes having greater SI values.

From each island, the individuals collected included larvae and adults of *D. punctatus*, and any pupae collected were maintained to obtain adults. Sampling sites were randomly distributed on an island, and we avoided sampling in the same or nearby sites. The specimens were stored individually in absolute ethanol.

DNA extraction, amplification and sequencing

We dissected each individual to remove the epidermis and internal contents and used the muscular tissue for the experiment. We isolated genomic DNA from muscular tissue using a Multisource Genomic DNA Miniprep Kit (Axygen Scientific, Inc., New York. USA). We amplified fragments of the mitochondrial DNA cytochrome c oxidase subunit I gene (mtDNA COI) using primers (SMCF2: 5′-CACAAAAGA TATTGGAACAT-3′ and SMCR1: 5′-GTGTTTAAATTTCGA TCAGT–3′). The length of the region amplified by these primers was approximately 600 bp. The authors designed the above primers in-house, because we failed to achieve amplification when using the universal primers of Lepidoptera. Polymerase chain reactions (PCRs) consisted of an initial denaturation step of 94°C for 3 min, followed by 34 cycles of denaturation at 94°C for 45 s, annealing at 44.7°C for 50 s and extension at 72°C for 50 s, followed by a final extension at 72°C for 5 min. PCR was conducted in a solution with a final volume of 50 µl, which contained 5 µl of 10× Taq buffer, 2 U of Taq polymerase, 0.5 mM each dNTP, 25 mmol primers and 2.5 µl of DNA template. Amplified fragments were electrophoresed and purified with gel extraction and then sequenced by Genscript (Genscript, Nan Jing, China).

Each *D. punctatus* sample was sequenced bi-directionally and assembled using the software ContigExpress. We used BLAST to align the obtained sequences with homologous sequences from GenBank to ensure amplification of the target sequences. We translated subject sequences using invertebrate mitochondrial codons to ensure that amplified fragments represented authentic mtDNA rather than numts. Sequences were aligned using the Clustal X 1.83 program (Chenna *et al.*, 2003) and manually adjusted.

Statistical analyses

We used DnaSP 5.0 (Rozas *et al.*, 2003) to calculate three population indices of genetic diversity: number of haplotypes (h), nucleotide diversity (π) and haplotype diversity. We used an ordinary least square regression to test for possible biases in the number of haplotypes at each island due to uneven sample sizes. Because we found no significant association between sample size and haplotype diversity, we used uncorrected haplotype numbers in all subsequent statistical analyses. We also report the number of haplotypes adjusted for sample size (h/N) for comparison. We used Arlequin 3.5 (Excoffier & Lischer, 2010) to calculate pairwise F-statistic (Fst) values as the measure of genetic differentiation between two different populations, with the significance tested using 10,000 permutations. Analysis of molecular variance (AMOVA) was also performed using Arlequin 3.5 (Du *et al.*, 2014).

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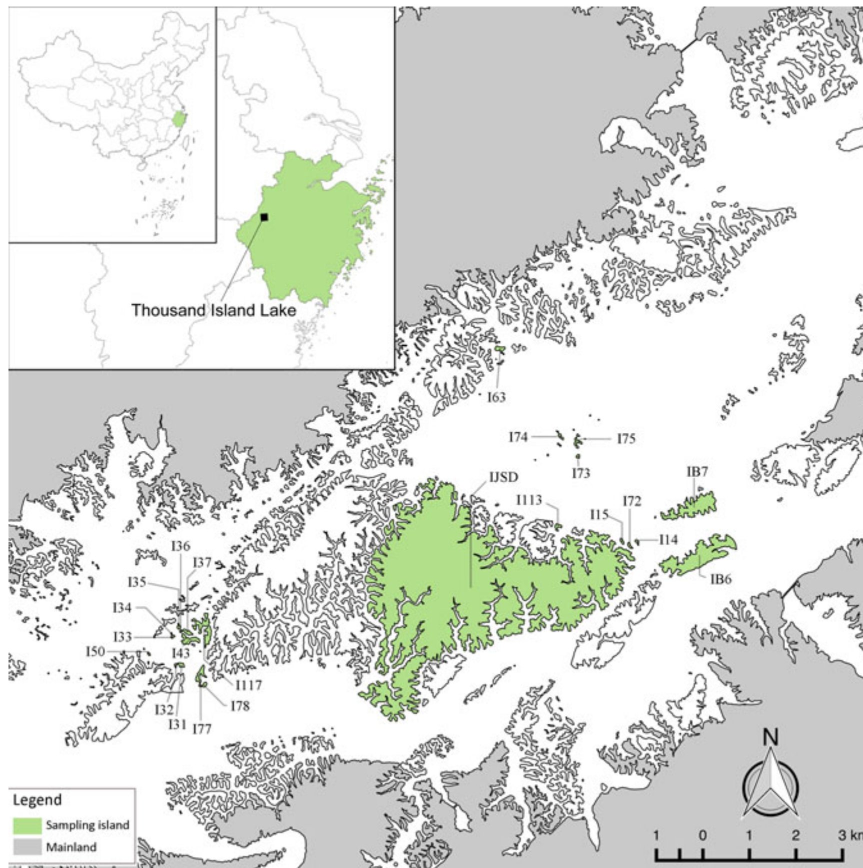

Fig. 1. Map showing geographical locations of the 23 islands sampled in TIL. Numbers refer to island IDs in table 1 and are used throughout the study.

Table 1. Parameters of the 23 islands sampled in this study.

Island ID	Area (ha)	Shape index	Isolation (m)
I14	0.4880	1.5862	121.1681
I15	0.5884	1.4105	77.688
I31	0.9270	1.3	14.535
I32	0.4880	1.4028	14.535
I33	0.4027	1.3038	15.2359
I34	0.0788	1.2571	15.2359
I35	0.5279	1.5111	28.2048
I36	0.1888	1.2407	10.8337
I37	1.3589	1.6276	10.8337
I43	4.0584	2.032	29.6475
I50	0.2890	1.2537	89.5415
I63	1.8190	1.8452	53.295
I72	0.6325	1.5657	65.5817
I73	0.4340	1.2439	135.66
I74	0.6228	1.6122	26.0411
I75	1.4217	2.2027	22.9535
I77	3.0339	2.287	16.15
I78	0.9225	1.6639	16.15
I113	1.1560	1.6493	59.5583
I117	9.7287	3.4134	71.2982
IB6	51.8885	3.3639	31.8118
IB7	29.0535	3.4207	67.3089
IJSD	1158.0853	9.5419	17.3941

To test island fragmentation for possible genetic effects, we used ordinary least square regression between the three island attributes, the logarithm of island area, isolation distance, and island SI, and the two indices of population genetic diversity, the number of haplotypes and haplotype diversity. We tested for a pattern of isolation by distance (IBD) across all islands by correlating a matrix of pairwise genetic distances (*F*st) between all islands with geographic distances using a Mantel matrix correspondence test. We used spatial AMOVA implemented in the program SAMOVA v. 1.0 (Dupanloup *et al.*, 2002) to define partitions of local populations that were maximally differentiated from one another based on our sequence data. The method is based on a simulated annealing procedure that maximizes the proportion of genetic variance that can be explained by differences between groups of populations. Our analyses were based on 100 simulated annealing steps and prior definition of the number of groups, *K*, ranging from 2 to 5. Strictly following the analysis assumptions, groups of only one population sample should have no genetic structure (Dupanloup *et al.*, 2002). For each analysis with increasing *K*, we examined the proportion of genetic variance due to differences between groups, *F*ct, and searched the range of *K* for which *F*ct was the largest and statistically significant. We constructed phylogenetic trees with 1000 bootstrap replications using MEGA 6.0 (Tamura *et al.*, 2011).

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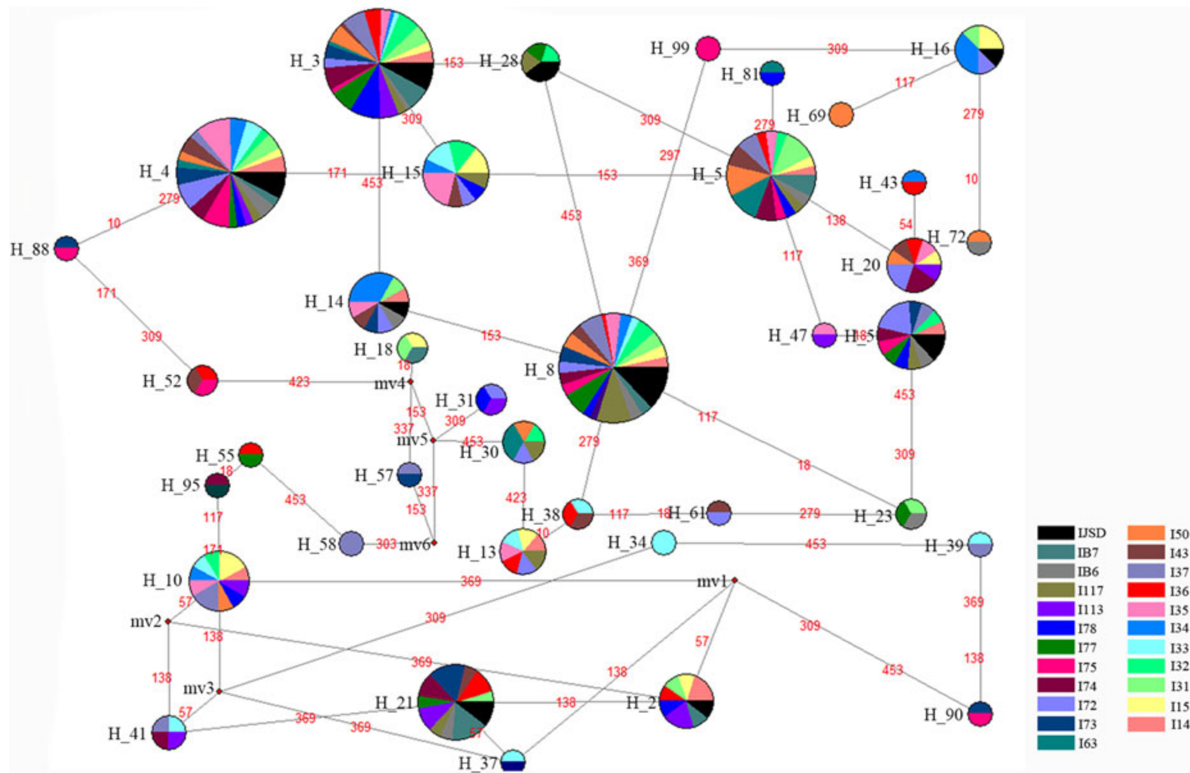

Fig. 2. Median-joining network of 37 main haplotypes based on analysis of Dendrolimus punctatus COI sequences. Each circle represents one haplotype and the circle size indicates haplotype frequency. Each circle is coloured based on the number of individuals from each island.

Table 2. Genetic index of the 23 populations of *Dendrolimus punctatus*.

Island ID	Haplotypes	Haplotype diversity (Hd)	Nucleotide diversity (π)
I14	14	0.95789	0.01027
I15	13	0.95906	0.01111
I31	14	0.93676	0.00957
I32	13	0.90909	0.00777
I33	16	0.97895	0.01284
I34	11	0.92398	0.01078
I35	14	0.93676	0.00659
I36	14	0.95425	0.01161
I37	11	0.88933	0.01032
I43	18	0.97835	0.01008
I50	14	0.93676	0.01051
I63	13	0.96667	0.00848
I72	14	0.94372	0.01256
I73	15	0.95652	0.00756
I74	12	0.92641	0.01248
I75	11	0.94167	0.00923
I77	14	0.90119	0.01015
I78	15	0.90476	0.00968
I113	16	0.96104	0.01353
I117	14	0.85375	0.00772
IB6	19	0.98268	0.01313
IB7	11	0.91053	0.01339
IJSD	19	0.89744	0.00991

For direct observation of the genetic connection among main haplotypes, we created a median-joining network (fig. 2) for 37 main haplotypes (these 37 haplotypes were detected in at least two individuals, and the other 104 haplotypes were detected in just one individual) using software Network 4.6 (Bandelt *et al.*, 1999).

Results

Population genetic diversity

We obtained 502 sequences for a 500 bp fragment of COI. These sequences included 37 variable sites of which 13 were singleton variable sites and 24 parsimony informative sites. All variable sites were transitional, with no insertions or deletions.

One hundred and forty-one distinct haplotypes were identified, which occurred in 28.09% of the total samples. One hundred haplotypes were unique to single populations and others were shared by at least two populations. The number of haplotypes (*h*) per population (adjusted for sample size) varied from 0.48 on island 37 (I37) to 0.86 on island B6 (IB6). The absolute number of haplotypes per population varied from 11 on islands 34, 37, 75 and B7 to 19 on islands B6 and JSD (table 2). Haplotype 3 was observed in all 23 populations at a rate between 5% (I33, I34, I43, IB6) and 32% (I78), and haplotype 8 was observed in 22 populations, with I63 the exception. Haplotype diversity (Hd) varied from 0.85375 on I117 to

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Table 3. Pairwise *F*st estimates among all populations of *Dendrolimus punctatus* included in this study (lower matrix).

	i14	i15	i31	i32	i33	i34	i35	i36	i37	i43	i50	i63	i72	i73	i74	i75	i77	i78	i113	i117	iB6	iB7	iJSD
i14		5.24	10.03	10.17	10.08	10.12	9.92	9.92	9.68	9.58	10.68	4.8	0.11	2.11	2.64	2.32	9.63	9.67	1.60	9.28	0.64	0.67	0.21
i15	−0.04069		9.72	9.86	9.77	9.81	9.61	9.59	9.37	9.28	10.36	4.70	0.09	1.94	2.47	2.15	9.31	9.37	1.28	8.97	0.87	0.91	0.02
i31	−0.01108	−0.01831		0.01	0.57	0.65	0.73	0.65	0.62	0.41	0.66	9.50	9.86	9.50	9.41	9.61	0.32	0.49	8.48	0.57	10.43	10.72	3.76
i32	−0.00670	−0.00574	−0.03285		0.56	0.63	0.74	0.67	0.66	0.44	0.54	9.59	10.00	9.63	9.53	9.72	0.47	0.60	8.61	0.67	10.55	10.86	3.92
i33	0.06791	0.04443	**0.13845**	**0.16910**		0.01	0.16	0.66	0.20	0.13	0.55	9.22	9.92	9.42	9.29	9.51	0.85	1.12	8.48	0.43	10.51	10.76	4.10
i34	−0.02372	−0.03673	−0.03024	−0.01762	**0.10038**		0.15	0.16	0.22	0.2	0.61	9.22	9.96	9.44	9.30	9.51	0.94	1.23	8.51	0.46	10.56	10.8	4.24
i35	0.03692	0.03850	−0.01084	−0.01633	**0.22997**	0.00479		0.01	0.04	0.1	0.8	8.99	9.76	9.22	9.07	9.29	0.92	1.26	8.3	0.25	10.36	10.58	4.05
i36	−0.02115	−0.02338	0.00332	0.00945	0.05356	−0.00964	0.05642		0.01	0.13	0.78	9.03	9.75	9.23	9.09	9.32	0.84	1.17	8.3	0.25	10.34	10.57	4.03
i37	−0.03350	−0.03458	0.00214	0.00516	0.04475	−0.00901	0.05281	−0.02751		0.09	0.84	8.88	9.53	9.04	8.92	9.12	0.64	0.95	8.05	0.06	10.1	10.21	3
i43	−0.03162	−0.03737	−0.02676	−0.02135	**0.08078**	−0.02619	0.01872	−0.02863	−0.02174		0.7	9.12	9.77	9.27	9.16	9.36	0.73	0.94	8.35	0.21	10.52	10.64	4
i50	−0.02693	−0.04198	−0.03004	−0.01582	**0.08316**	−0.03633	0.02400	−0.01255	−0.02067	−0.02962		9.86	10.51	10.04	9.92	10.14	1.09	1.22	9.08	1.08	11.09	11.35	4.77
i63	0.04022	0.02572	0.03924	0.04517	**0.14550**	**0.06265**	**0.09696**	0.03042	0.04519	0.00965	0.03861		4.93	2.69	2.19	1.99	9.4	9.67	3.88	8.57	5.68	4.78	3.44
i72	−0.01905	−0.02222	0.03345	0.04732	0.00908	0.00886	**0.09731**	−0.03349	−0.02470	−0.00792	0.00353	0.04670		2.01	2.60	2.19	9.48	9.54	1.45	9.13	0.83	0.74	4.91
i73	0.02196	0.01337	−0.02557	−0.02398	**0.18376**	−0.00482	−0.01046	0.04057	0.03791	−0.01193	0.00004	**0.05864**	**0.07901**		0.46	0.04	9.18	9.3	1.44	8.57	2.77	2	1.69
i74	−0.00583	−0.02095	0.00194	0.02219	0.03742	−0.01278	0.05898	−0.02750	−0.01724	−0.01869	−0.00863	0.06051	−0.02046	0.03518		0.28	9.05	9.23	1.84	8.52	3.5	2.5	1.6
i75	0.00907	−0.01013	−0.02727	−0.01634	**0.14050**	−0.01759	−0.00950	0.01061	0.02087	−0.01947	−0.01380	0.02084	0.03475	−0.01590	0.01000		9.2	9.37	1.67	8.66	2.97	2.24	1.86
i77	−0.03375	−0.02111	0.01423	0.01215	0.06290	−0.00634	**0.06767**	−0.01828	−0.02916	−0.01221	−0.00730	**0.06726**	−0.01472	0.04688	0.00393	0.04515		0.07	8.16	0.6	10.22	10.28	3.3
i78	−0.00761	−0.00912	−0.02633	−0.01684	**0.12275**	−0.01408	−0.01269	0.00025	−0.00661	−0.01904	−0.00975	0.03107	0.02133	−0.00925	−0.00071	−0.02392	0.01080		8.37	0.62	10.25	10.46	2.43
i113	0.02718	0.01886	**0.09799**	**0.12282**	−0.02237	0.06215	**0.18805**	0.01148	0.01505	0.04898	0.05127	**0.11244**	−0.02119	**0.15244**	0.01252	**0.11404**	0.01777	**0.09046**		7.67	2.28	2.21	0.53
i117	−0.01225	−0.00865	−0.02529	−0.03347	**0.15992**	−0.01216	0.00772	0.00563	0.00137	−0.02012	−0.02197	**0.05989**	0.04602	−0.01143	0.02751	0.00741	0.00726	0.00183	**0.11311**		9.81	10.03	3.38
iB6	−0.00556	−0.01642	0.05435	**0.07904**	−0.00194	0.01246	**0.13552**	0.00963	0.00042	0.01761	0.01133	**0.09775**	−0.02113	**0.10278**	−0.00029	**0.06965**	0.00044	0.05978	−0.02336	**0.07019**		0.72	3.21
iB7	−0.01038	−0.01884	0.02837	0.05310	0.01348	0.00125	**0.10046**	−0.02714	−0.01150	−0.00420	0.00378	0.06122	−0.03875	**0.08014**	−0.03148	0.03492	−0.00134	0.02453	−0.02120	0.05418	−0.02549		3.21
iJSD	−0.01511	−0.01984	−0.01997	−0.00875	**0.10635**	−0.02254	0.02853	−0.00535	−0.00827	−0.02122	−0.02499	**0.06468**	0.01520	0.00397	−0.00929	0.00050	0.00228	−0.00218	**0.06791**	−0.01281	0.02822	0.01150	

Significant pairwise comparisons ($P < 0.05$) are indicated in bold. Pairwise geographical distances among sampling sites are in the upper matrix (km). Locality names are the same as those in fig. 1.

0.98268 on IB6, with the global Hd 0.935 (±0.006). Nucleotide diversity by site (π) varied from 0.00659 on I35 to 0.01353 on I113.

Pairwise *F*st values (a measure of connectivity between pairs of sites) ranged from 0.00004 to 0.22997 (generally, *F*st is a value ranged from 0 to 1; therefore, we used the absolute value of the value of the software calculation). The highest value was between I33 and I35; whereas the lowest value was between I50 and I73. Although most pairwise comparisons indicated relatively low differentiation among populations, 37 of the pairwise comparisons (6.99%) were significantly different from zero (bold in table 3). Most of the significant *F*st comparisons occurred between I33 and other populations. Twelve of the significant comparisons occurred between I33 and other populations, whereas nine occurred between I63 and other populations and between I113 and other populations (table 3).

The analysis of variance and the correlation analysis for the effect of island area, shape and isolation showed no significant differences for haplotype diversity (Hd), despite a negative trend for low Hd in the large and more complex fragments. The number of haplotypes (*h*) was significantly and positively correlated with the logarithm of island area and island SI. By contrast, the number of haplotypes (*h*) did not vary significantly with isolation. However, when the island JSD was not included in the analysis, no significant correlation occurred between island area and haplotypes or between island shape and haplotypes.

Spatial analyses

We found no evidence of IBD in the Mantel test that included all samples (fig. 3). The SAMOVA identified maximally differentiated groups in our sample. The interaction between *F*ct and *F*st was expected and is one of the difficulties of using *F*ct to define the 'real' number of differentiated groups. The *F*ct estimated at high values of *K* remained significant but increased incrementally as differentiation within each group decreased (Dupanloup *et al.*, 2002). Therefore, we compared the inferred population structure in the range of K that showed the highest significant values for *F*ct (table 4). Our results showed a hierarchical grouping arrangement as *K* increased from 2 to 5 (table 4). Results of SAMOVA showed that, with the increase of *K*, the population samples that formed independent groups were the populations most distant from the central IJSD (largest sample island) and nearest to the mainland in a different direction. Additionally, AMOVA showed that a relatively high proportion (89.1%) of the total genetic variance was attributable to variation within populations (table 5).

We constructed a minimum-evolution tree (Fig. 4) and a neighbour-joining tree (Fig. 5), with *Dendrolimus kikuchii* (Lepidoptera: Lasiocampidae) as the out-group, to explore the relationship between the distribution of haplotypes and geographic location. Haplotypes that were on adjacent islands, even the same island, were assigned to two groups, such as Hap14 (appeared on I43, I34 and I35) and Hap65 (appeared on I43), whereas those haplotypes that distributed most distant were similar in phylogenetic trees, such as Hap112 (appeared on I78) and Hap4 (appeared on IB6 and IB7). The median-joining network analysis (fig. 5) also indicated the similar result, Hap8 played a crucial role in connecting other haplotypes and each haplotype connected by the other haplotype, the results indicated genetic connection

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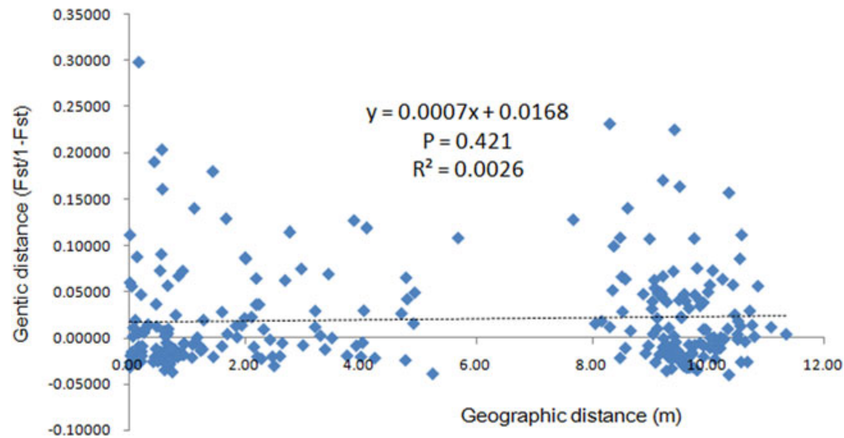

Fig. 3. Scatter plot of pairwise genetic distance vs. geographical distance for all 23 islands included in this study.

Table 4. Results of fixation indices corresponding to the groups of populations inferred by SAMOVA for the 23 *Dendrolimus punctatus* populations tested for variation in the mtDNA cytochrome c oxidase subunit I gene.

Groups	*F*-values	*P*-value	% Var
Two groups	**Fct = 0.09269**	**0.04497**	9.27
1. I33	*F*sc = 0.01121	0.00684	1.02
2. Remaining population	*F*st = 0.10286	0.01271	89.71
Three groups	**Fct = 0.05081**	**0.02542**	5.08
1. I63	*F*sc = 0.01139	0.00880	1.08
2. I113	*F*st = 0.06162	0.01173	93.84
3. Remaining population			
Four groups	**Fct = 0.04529**	**0.01760**	5.08
1. I63	*F*sc = 0.00852	0.00196	1.08
2. I113	*F*st = 0.05342	0.01075	93.84
3. IB6			
4. Remaining population			
Five groups	**Fct = 0.06324**	**0.00000**	6.32
1. I33	*F*sc = −0.00396	0.01662	−0.37
2. I63	*F*st = 0.05952	0.01173	94.05
3. I113 and IB6			
4. I75			
5. Remaining population			

among main haplotypes, whereas no geographic distribution structure among different haplotypes.

Discussion

We found a significant decrease in the number of haplotypes on small islands with less area (fig. 6), which indicated that small island area had a potential negative effect on genetic diversity of these island populations. Fewer haplotypes were maintained on the small islands likely because of genetic drift or random loss of haplotypes on small fragments with reduced effective population size and colonization rate. We also found a significant increase in the number of haplotypes on islands with a high SI, indicating that islands with a more complex shape supported higher genetic diversity. However, the significant correlations described above were no longer significant when island JSD was not included (fig. 7).

In addition to the effects of habitat area and shape, the genetic diversity of a species is also affected by species-typical characteristics, such as habitat specialization, vagility and ecological tolerance, which influence the susceptibility of individual species to habitat fragmentation (Sumner *et al.*, 2004; Peakall & Lindenmayer, 2006; Louy *et al.*, 2007). Species with high dispersal ability and with a high number of habitat patches have a better chance of maintaining gene flow and panmixia; and low dispersal capacity and high number of accessible fragments result in a pattern of IBD. However, low dispersal capacity and low habitat availability result in a species becoming isolated, and with gene flow absent, drift is aggravated independently in each population (Louy *et al.*, 2007; Marianna *et al.*, 2009). The value of *F*st in our study also confirmed a certain degree of gene flow between each island population (table 3). Additionally, we found no significant correlation between genetic distance and geographic distance (fig. 3), which is consistent with the high dispersal capacity of *D. punctatus*, allowing the moths to cross over the isolation of water among islands. This result suggested that the dispersal capacity of the species could weaken or counteract the isolation effect of fragmentation by removing the genetic barriers, which would then prevent regional-scale spatial genetic structure (Wang *et al.*, 2012).

Table 5. Analysis of molecular variance (AMOVA) for the 23 populations of *Dendrolimus punctatus*.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices	*P*-value
Among groups	1	13.429	0.26701 Va	9.27	*F*ct:0.09269	0.00684 ± 0.00231
Among populations within groups	21	67.727	0.02929 Vb	1.02	*F*sc:0.01121	0.05474 ± 0.00736
Within populations	479	1237.966	2.58448 Vc	89.71	*F*st:0.10286	0.04399 ± 0.00657
Total	501	1319.122	6.82397			

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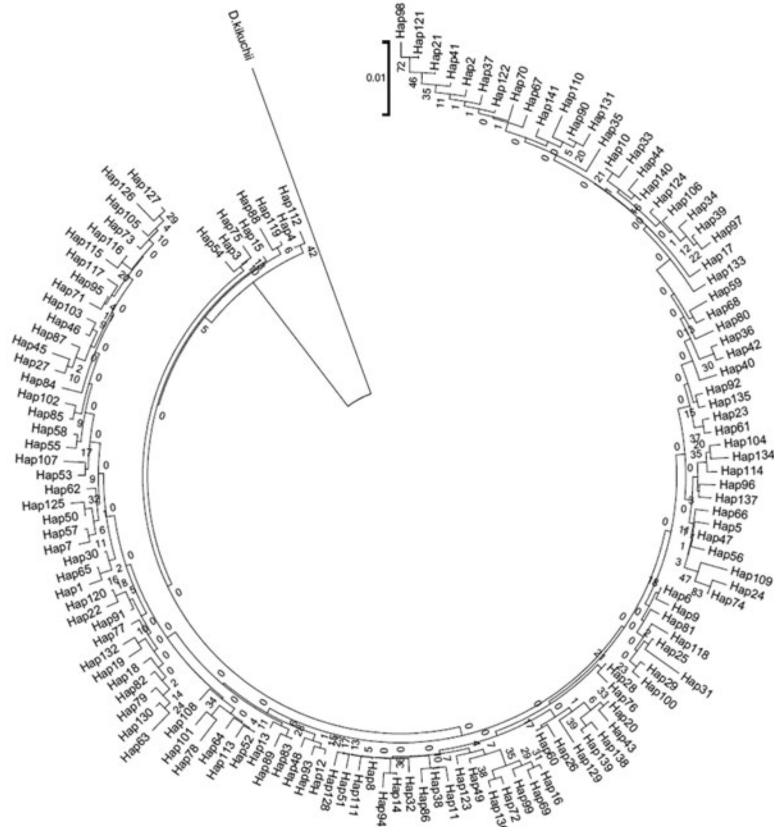

Fig. 4. Molecular phylogenetic trees of Dendrolimus punctatus using Minimum-Evolution (ME) based on COI gene sequence data.

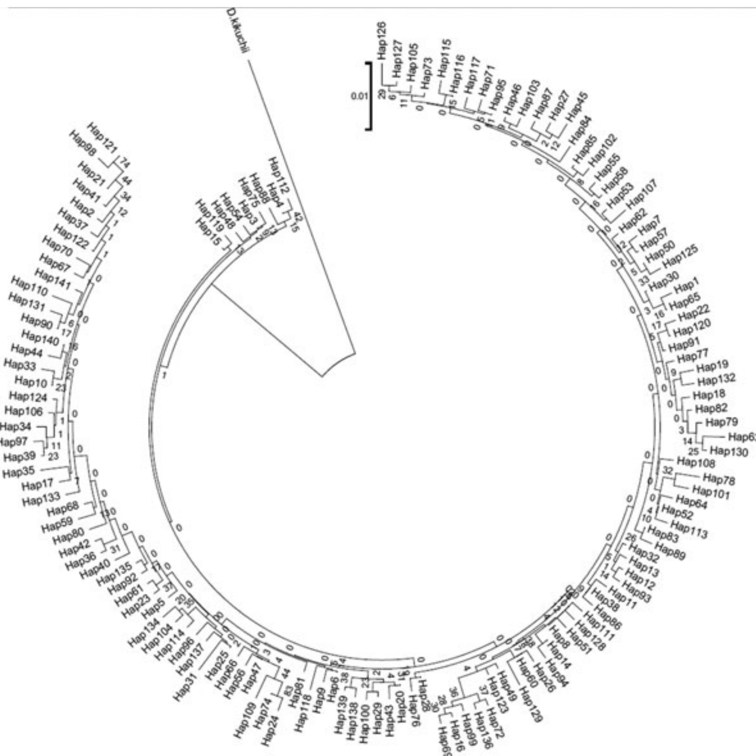

Fig. 5. Molecular phylogenetic trees of Dendrolimus punctatus using neighbour-joining (NJ) based on COI gene sequence data.

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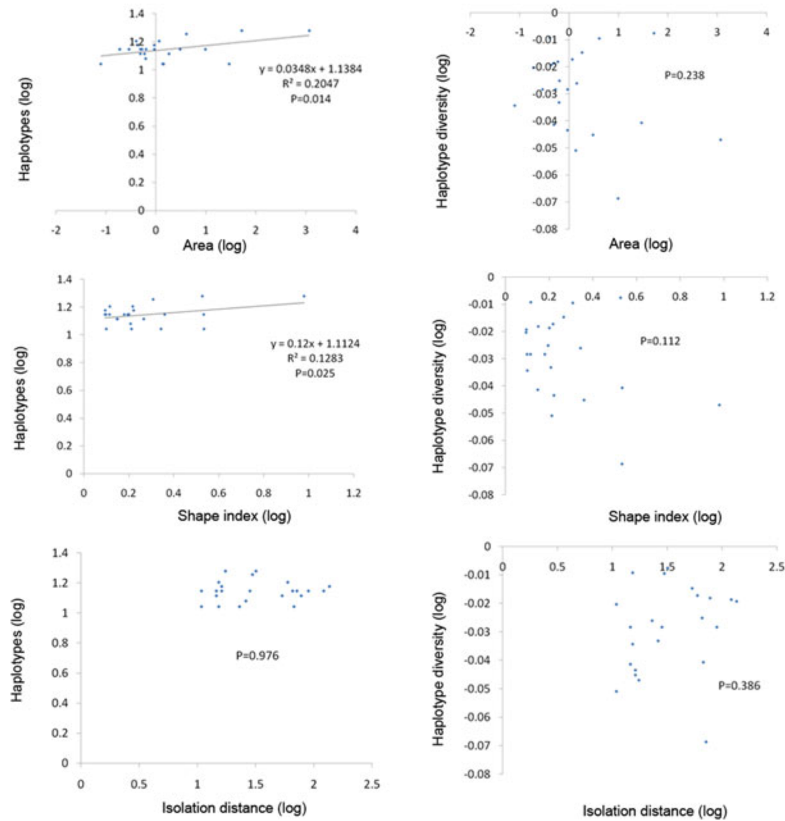

Fig. 6. Variation in the number of haplotypes and haplotype diversity estimated from mtDNA cytochrome c oxidase subunit I gene sequences of Dendrolimus punctatus in relation to the area, shape index, and isolation of the islands (with island JSD).

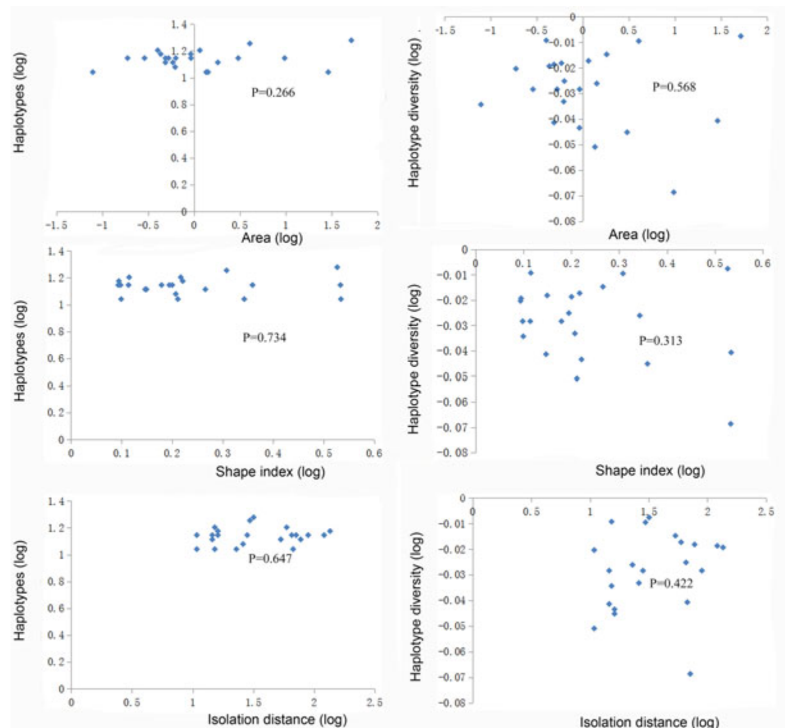

Fig. 7. Variation in the number of haplotypes and haplotype diversity estimated from mtDNA cytochrome c oxidase subunit I gene sequences of Dendrolimus punctatus in relation to the area, shape index, and isolation of the islands (without island JSD).

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Another factor affecting pine moth migration and spread is the distribution and density of host trees (primarily *Pinus* spp.). The primary vegetation on the islands of TIL is the natural secondary forest dominated by *Pinus massoniana*, which is mixed with many broad-leaved trees and shrub species (Wang *et al.*, 2010). Thus, *D. punctatus* populations on each island faced almost no selection pressure from the environment or comestibles. Therefore, the spread of *D. punctatus* was not influenced by environment or comestibles. Collectively, these factors (high dispersal capacity, high habitat availability, low selection pressure and sufficient generations of isolation) resulted in the completely random migration of *D. punctatus* from island to island, thus formed the present genetic structure and the undefined genetic diversity distribution pattern of *D. punctatus* on the islands of TIL.

Our results revealed that the geographical isolation and current distribution of fragments did not completely obstruct gene flow and did not lead to significant genetic differentiation among populations of *D. punctatus* on different islands. Nevertheless, reductions in genetic diversity and changes in haplotype diversity due to genetic drift can occur rapidly when migration is curtailed and population sizes are small (Lacy, 1987; Peakall & Lindenmayer, 2006). Our results emphasize that the primary effect of fragmentation on the genetic diversity of *D. punctatus* in TIL resulted from fragments small in area, which underscores that those species with relatively high dispersal capacities and high tolerance to disturbed environments can also suffer the negative genetic effects of habitat fragmentation (Peakall & Lindenmayer, 2006).

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

Bandelt, H.J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology & Evolution* **16**, 37.

Bao, Y. (2013) Effects of islanding on the genetics of *Niviventer confucianus* (Mamalia: *Rodentia: Muridae*) populations in the Thousand Island Lake region. *Journal of Natural History* **47**, 2583–2598.

Bender, D.J., Contreras, T.A. & Fahrig, L. (1998) Habitat loss and population decline: a meta-analysis of the patch size effect. *Ecology* **79**, 517–533.

Bevers, M. & Flather, C.H. (1999) Numerically exploring habitat fragmentation effects on populations using cell-based coupled map lattices. *Theoretical Population Biology* **55**, 61–76.

Bowers, M.A. & Matter, S.F. (1997) Landscape ecology of mammals: relationships between density and patch size. *Journal of Mammalogy* **78**, 999–1013.

Bukey, T.V. (1995) Extinction rates in archipelagos: implications for populations in fragmented habitats. *Conservation Biology* **9**, 527–541.

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T.J., Higgins, D.G. & Thompson, J.D. (2003) Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Research* **31**, 3497.

Collinge, S.K. (1996) Ecological consequences of habitat fragmentation: implications for landscape architecture and planning. *Landscape and Urban Planning* **36**, 59–77.

Collinge, S.K. & Palmer, T.M. (2002) The influences of patch shape and boundary contrast on insect response to fragmentation in California grasslands. *Landscape Ecology* **17**, 647–656.

Crooks, K.R., Suarez, A.V., Bolger, D.T. & Soulé, M.E. (2001) Extinction and colonization of birds on habitat islands. *Conservation Biology* **15**, 159–172.

Cumming, G. (2002) Habitat shape, species invasions, and reserve design: insights from simple models. *Ecology & Society* **6**, 840–842.

Dobson, A.P., Bradshaw, A.D. & Baker, A.J.M. (1997) Hopes for the future: restoration ecology and conservation biology. *Science* **277**, 515–522.

Du, Y.Z., Tang, X.T., Wang, L.P., Shen, Y. & Chang, Y.W. (2014) Genetic differentiation of geographical populations of *Liriomyza sativae* (Diptera: Agromyzidae) in China based on mitochondrial COI gene sequences. *Mitochondrial DNA* **2014**, 1–5.

Dupanloup, I., Schneider, S. & Excoffier, L. (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* **11**, 2571.

Ewers, R.M. & Didham, R.K. (2007) The effect of fragment shape and species' sensitivity to habitat edges on animal population size. *Conservation Biology* **21**, 926.

Excoffier, L. & Lischer, H.E. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564–567.

Fagan, W.F. (2002) Connectivity, fragmentation, and extinction risk in dendritic metapopulations. *Ecology* **83**, 3243–3249.

Fahrig, L. (2003) Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology, Evolution and Systematics* **34**, 487–515.

Fei, H., Wang, H., Kong, X., Zhang, Z., Zhang, S. & Song, X. (2014) Selection and prediction of meteorological factors correlated with *Dendrolimus punctatus* outbreak. *Journal of Northeast Forestry University* **42**, 136–140.

Franklin, A.B., Noon, B.R. & George, T.L. (2002) What is habitat fragmentation? pp. 20–29. *in* George, T. L. and Dobkin, D. S. (*Eds*) *Effects of Habitat Fragmentation on Birds in Western Landscapes: Contrasts with Paradigms From the Eastern United States. Studies in Avian Biology* **25**, 20–29.

Game, M. (1980) Best shape for nature reserves. *Nature* **287**, 630–632.

Goossens, B., Sharma, R., Othman, N., Kun-Rodrigues, C., Sakong, R., Ancrenaz, M., Ambu Laurentius, N., Jue Nathaniel, K., O'Neill, R.J., Bruford, M.W & Chikhi, L. *et al.* (2016) Habitat fragmentation and genetic diversity in natural populations of the Bornean elephant: implications for conservation. *Biological Conservation* **196**, 80–92.

Hamazaki, T. (1996) Effects of patch shape on the number of organisms. *Landscape Ecology* **11**, 299–306.

Hames, R.S., Rosenburg, K.V., Lowe, J.D. & Dhondt, A.A. (2001) Site reoccupation in fragmented landscapes: testing predictions of metapopulation theory. *Journal of Animal Ecology* **70**, 182–190.

Hanski, I. (1998) Metapopulation dynamics. *Nature* **396**, 41–49.

Henle, K., Lindenmayer, D.B., Margules, C.R., Saunders, D.A. & Wissel, C. (2004) Species survival in fragmented landscapes: where are we now? *Biodiversity and Conservation* **13**, 1–8.

https://doi.org/10.1017/S0007485318000172 Published online by Cambridge University Press

Hoffmeister, T.S., Lem, V., Biere, A., Holsinger, K. & Filser, J. (2005) Ecological and evolutionary consequences of biological invasion and habitat fragmentation. *Ecosystems* **8**, 657–667.

Jia, X., He, Z., Weiser, M.D., Yin, T., Akbar, S., Kong, X., Tian, K., Jia, Y., Lin, H., Yu, M. & Tian, X. (2016) Indoor evidence for the contribution of soil microbes and corresponding environments to the decomposition of *Pinus massoniana*, and *Castanopsis sclerophylla*, litter from Thousand Island Lake. *European Journal of Soil Biology* **77**, 44–52.

Johansson, M., Primmer, C.R. & Merilä, J. (2004) Does habitat fragmentation reduce fitness and adaptability? A case study of the common frog (*Rana temporaria*). *Molecular Ecology* **16**, 2693.

Krauss, J., Schmitt, T., Seitz, A., Steffan-Dewenter, I. & Tscharntke, T. (2004) Effects of habitat fragmentation on the genetic structure of the monophagous butterfly *Polyommatus coridon* along its northern range margin. *Molecular Ecology* **13**, 311–320.

Lacy, R.C. (1987) Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conservation Biology* **1**, 143–158.

Laurance, W.F. & Yensen, E. (1991) Predicting the impacts of edge effects in fragmented habitats. *Biological Conservation* **55**, 77–92.

Leisnham, P.T. & Jamieson, I.G. (2002) Metapopulation dynamics of a flightless alpine insect *Hemideina maori* in a naturally fragmented habitat. *Ecological Entomology* **27**, 574–580.

Louy, D., Habel, J.C., Schmitt, T., Assmann, T., Meyer, M. & Müller, P. (2007) Strongly diverging population genetic patterns of three skipper species: the role of habitat fragmentation and dispersal ability. *Conservation Genetics* **8**, 671–681.

Marianna, D., Jeanpaul, M., Joãos, M. & Kellyr, Z. (2009) Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. *Biological Conservation* **142**, 1560–1569.

Peakall, R. & Lindenmayer, D. (2006) Genetic insights into population recovery following experimental perturbation in a fragmented landscape. *Biological Conservation* **132**, 520–532.

Ramirez, M.G. & Haakonsen, K.E. (1999) Gene flow among habitat patches on a fragmented landscape in the spider *Argiope trifasciata* (Araneae: araneidae). *Heredity* **83**, 580–585.

Ranta, P., Blom, T., Niemela, J., Joensuu, E. & Siitonen, M. (1998) The fragmented Atlantic rain forest of Brazil: size, shape and distribution of forest fragments. *Biodiversity and Conservation* **7**, 385–403.

Reed, D.H. & Frankham, R. (2003) Correlation between fitness and genetic diversity. *Conservation Biology* **17**, 230–237.

Rozas, J., Sánchez-Delbarrio, J.C., Messeguer, X. & Rozas, R. (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**, 2496–2497.

Schmitt, T. & Seitz, A. (2002) Influence of habitat fragmentation on the genetic structure of *Polyommatus coridon* (Lepidoptera: Lycaenidae): implications for conservation. *Biological Conservation* **107**, 291–297.

Schoereder, J.H., Sorbrino, T.G., Ribas, C.R. & Campos, R.B.F. (2004) Colonization and extinction of ant communities in a fragmented landscape. *Austral Ecology* **29**, 391–398.

Sumner, J., Jessop, T., Paetkau, D. & Moritz, C. (2004) Limited effect of anthropogenic habitat fragmentation on molecular diversity in a rain forest skink, *Gnypetoscincus queenslandiae*. *Molecular Ecology* **13**, 259–269.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) Mega5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology & Evolution* **28**, 2731–2739.

Tilman, D., May, R.M., Lehman, C.L. & Nowak, M.A. (1994) Habitat destruction and the extinction debt. *Nature* **371**, 65–66.

Van Dongen, S.V., Backeljau, T., Matthysen, E. & Dhondt, A.A. (1998) Genetic population structure of the winter moth (*Operophtera brumata I.*) (lepidoptera, geometridae) in a fragmented landscape. *Heredity* **80**, 92–100.

Wang, R., Compton, S.G. & Chen, X.Y. (2011) Fragmentation can increase spatial genetic structure without decreasing pollen-mediated gene flow in a wind-pollinated tree. *Molecular Ecology* **20**, 4421–4432.

Wang, R., Compton, S.G., Shi, Y. & Chen, X.Y. (2012) Fragmentation reduces regional-scale spatial genetic structure in a wind-pollinated tree because genetic barriers are removed. *Ecology & Evolution* **2**, 2250–2261.

Wang, Y.P., Bao, Y.X., Yu, M.J., Xu, G.F. & Ping, D. (2010) Nestedness for different reasons: the distributions of birds, lizards and small mammals on islands of an inundated lake. *Diversity & Distributions* **16**, 862–873.

Watson, D.M. (2002) A conceptual framework for studying species composition in fragments, islands and other patchy ecosystems. *Journal of Biogeography* **29**, 823–834.

Wood, B.C. & Pullin, A.S. (2002) Persistence of species in a fragmented urban landscape: the importance of dispersal ability and habitat availability for grassland butterflies. *Biodiversity and Conservation* **11**, 1451–1468.

Yu, M. & Mcgeoch, M. (2012) Richness and composition of plants and birds on land-bridge islands: effects of island attributes and differential responses of species groups. *Journal of Biogeography* **39**, 1124–1133.

https://doi.org/10.1017/S0007485318000172 Published online by Cambridge University Press