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Impacts of forest fragmentation on the genetic diversity and population structure of *Pachycondyla obscurans* in Sabah, Malaysian Borneo

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ABSTRACT. The impact of forest fragmentation on genetic diversity of *Pachycondyla obscurans*, a predatory forest-specialist ant species, was investigated in six forest locations using Amplified Fragment Length Polymorphism (AFLP) fingerprinting. There was little difference in genetic diversity between three large forest locations (two locations in continuous forest, plus one 120,000 ha fragment) and three small forest fragments (151–320 ha). Populations from two locations approximately 60 km apart in continuous forest showed little genetic differentiation, but genetic differences between all other locations (35–164 km apart), which are separated by oil palm plantations, were much greater. There was no significant relationship between geographical distance and genetic distance (pairwise F_{ST} values) between locations. Overall, we conclude that the genetic diversity of *P. obscurans* has not been affected by habitat fragmentation over the past fifty years, but locations separated by forest were more genetically similar than those separated by agricultural areas. This implies that an inhospitable intervening matrix may prevent ant dispersal, and may result in genetic erosion of insect populations in the longer term.

Keywords: Forest fragmentation, ants, *Pachycondyla obscurans*, genetic diversity, Sabah, Malaysia

INTRODUCTION

Forest fragmentation is recognized as one of the major threats to biodiversity (Laurance *et al*. 2000; Brook *et al*. 2002). In tropical regions, fragmentation of forest arises from many causes including land-use changes due to agriculture, urbanisation, and commercial logging. As a result of fragmentation, previously continuous tracts of forest are divided into fragments, and these fragments become habitat "islands" isolated within inhospitable landscape matrices. True oceanic islands that are small and isolated are known to support fewer species compared with larger islands (MacArthur & Wilson 1967), and similar patterns are evident in habitat "island" fragments (Rosenzweig & Clark 1995). Forest fragmentation and isolation of remnants is occurring in most tropical regions, often coinciding with the locations of biodiversity

"hotspots" (Myers 2003), and so contributing to global loss of biodiversity. Ecological impacts of forest fragmentation and the resulting isolation of populations leads to changes in the structure of the forest and causes disruption of biological processes and ecosystem functioning within fragments (Didham *et al.* 1996); thus, increasing the risk of extinction of populations (Vasconselos *et al*. 2006). Fragmentation can result in changes to abiotic and biotic factors, resulting in reduced population sizes within fragments, lower immigration rates of native species, more pronounced edge effects, increased immigration of alien species, and greater incidence of natural and human-caused forest disturbance (Turner 1996). The detrimental impacts of these effects on biodiversity mean there is an urgent need to study the consequences of forest fragmentation on populations of forest species in order to develop more sustainable forest management and effective conservation plans.

Forest fragmentation is commonly observed in many tropical regions, and on Borneo, recent forest fragmentation has arisen primarily from the conversion of forest into oil palm (*Elaeis guineensis* Jacq.) plantations (McMorrow & Talip 2001; Brühl & Eltz 2010). Insects are one of the most diverse groups of animals on Earth, and are especially diverse in tropical forests, but information on the effects of forest fragmentation on insect populations in this region are limited (Benedick *et al*. 2006). Previous studies on the responses of insects to tropical forest fragmentation have focused mainly on the effects of forest remnant size and isolation on community composition, and species richness and abundance (Klein 1989, De Souza & Brown 1994, Brown & Hutchings 1997, Didham 1997, Davies & Margules 1998, Kitching *et al.* 2000, Brühl *et al*. 2003, Benedick *et al*. 2006). For example, forest fragmentation affects the composition of ant assemblages due to increased colonisation of small fragments by generalist ants that can persist in the surrounding landscape matrix (Brühl *et al*. 2003; Schoereder *et al*. 2004; Bickel *et al*. 2006; Peters & Okalo 2009). Other studies on insects have shown that the species richness and diversity of butterflies is positively related to forest remnant size and negatively related to fragment isolation (Benedick *et al*. 2006). Although species richness is usually reduced in small fragments, these studies on butterflies have shown that small fragments can be important for conserving beta diversity at the landscape scale if small fragments support unique species not found elsewhere (Benedick *et al*. 2006).

Previous studies have also examined changes in genetic characteristics of ant populations in forest fragments in Sabah, Borneo (Bickel *et al.* 2006). Such information is important for understanding how fragmentation may lead to local adaptation arising from reduced gene flow between fragments (Hartl & Clark 1989; Templeton *et al*. 2001). In addition, changes in genetic diversity following fragmentation may lead to genetic erosion and genetic drift in populations in fragmented habitats, which may eventually increase the risk of local extinction for species at these sites (Bickel *et al.* 2006; Benedick *et al*. 2007). These previous genetic studies in Sabah on ants and butterflies (Benedick *et al.* 2007; Bickel *et al.* 2006) have shown that nucleotide diversity was reduced in fragmented habitats with increasing isolation (butterflies) and decreasing habitat size (ants). However, Benedick *et al.* (2007) concluded that overall changes in genetic diversity following forest fragmentation were relatively slight in butterflies, which could have been due to the relatively large sizes of forest fragments studied, or due to the short time since fragmentation had occurred. Lack of genetic changes might also imply sufficient gene flow and dispersal of butterflies among fragments. However, dispersal among fragments was unlikely because the Satyrinae butterfly species studied by Benedick *et al*. (2007), *Mycalesis orseis*, is dependent on closed-canopy forest (Hamer *et al.* 2003) and has not been recorded outside forest habitats or in plantations (Benedick *et al*. 2006). In the case of leaf-litter ants, Bickel *et al*. (2006) recorded a significant reduction in genetic diversity following fragmentation in a forest specialist species *Pheidole annexus*, indicating that forest fragmentation resulted in genetic erosion, although this effect was not observed in a second study ant species. By contrast, Benedick *et al.* (2007) suggested that isolation of fragments was more important than fragment size for genetic diversity of butterflies. These two studies in Sabah indicate that different taxa may respond differently to fragmentation. These findings may be related

to differences in sizes and placements of habitat fragments being studied, as well as differences among taxa in their ability to occupy matrix habitats and disperse among remaining fragments. Thus, there is a need for further investigations of the genetic consequences of fragmentation on species to better understand whether or not genetic erosion is likely to be an important factor affecting species persistence in fragments.

Ant assemblages provide an important element to study the effects of forest fragmentation on genetic diversity since they include many habitat-specialist species that are especially sensitive to habitat fragmentation (Boswell *et al.* 1998). Ants are an ecologically important group of insects in tropical forest due to their high abundance and the important ecological functions they perform as ecosystem engineers (Hölldobler & Wilson 1990). Many ant species have limited dispersal abilities leading to genetic erosion, genetic drift, and inbreeding in small populations in fragmented forest (Bickel *et al.* 2006). Inbreeding may ultimately lead to a higher susceptibility of extinction for populations (Frankham 1998), and some previous studies imply that genetic diversity of ants is reduced following forest fragmentation (Brühl *et al.* 2003).

There are several molecular genetic techniques available for quantifying genetic diversity within populations, and in this study, genetic diversity was measured using Amplified Fragment Length Polymorphism (AFLP) (Vos *et al*. 1995). The ability of AFLP to produce bands (DNA fragments) from numerous loci (>1000) at moderate financial costs has made it ideal for evaluating genetic diversity within populations, and for studying population genetic structure (Lynch 2002; Luikart *et al*. 2003). Some previous studies have used AFLPs for investigating the effects of habitat fragmentation on plants (Stanton *et al*. 2009) and insects (Monaghan *et al*. 2002), and in tropical regions, they have been used to study the genetic diversity of *Aedes aegypti* populations in Mexico (Ravel *et al*. 2001).

The present study examined the effects of forest fragment size and isolation on genetic variability of a forest-specialist species of ground-dwelling ant *Pachycondyla obscurans* (Walker, 1859) from six populations in Sabah (Malaysian Borneo). *Pachycondyla obscurans* is a leaf-litter forest specialist species that occurs relatively commonly at forest sites in Sabah. There are few studies on the ecology of *P. obscurans*, but it is classified as a small-sized species (workers are approximately 4 mm in length), with nests usually constructed in soil or rotten wood. The species-group occurs in the Australasian, Oriental and Ethiopian regions (Yamane 2007), and is synonymous with *Brachyponera* (Bolton 1995), whose workers are small, solitary epigaeic generalist predators and scavengers in forest litter, suggesting the species is likely to be adversely affected by forest fragmentation. *Pachycondyla obscurans* shows marked intra-sexual dimorphism between workers and queens, with the workers having completely lost their reproductive organs and queens having a large number of ovarioles (Christian Peeters, Laboratoire Ecologie & Evolution, Université Pierre et Marie Curie; pers. comm., 7 January 2011).

This ant species was chosen for genetic study because of its restricted habitat range as a specialist predator, and because this species has not been recorded outside forest habitats in the surrounding oil palm plantations (e.g., Brühl & Eltz 2010). Therefore, *P. obscurans* populations in forest fragments are likely to be isolated from one another with limited gene flow between fragments. In this study, we investigated changes in genetic diversity among populations of *P. obscurans* following forest fragmentation, and we tested the hypothesis that genetic diversity is lower in smaller fragments and related to geographical distance between sites.

MATERIALS AND METHODS

Sample collection

Twenty workers of *P. obscurans* were sampled from each of the six study locations in Sabah between June to September 2006 and October to November 2007. Ants were sampled along 2-km transects at each study location. A total of five sites were sampled along each transect using Winkler leaf-litter extraction techniques. Sites along transects were 500 m apart to ensure that samples were of individuals from different colonies. At each site, five 1 m^2 quadrats were sampled, that were each more than 10 m apart

to ensure that sampled individuals were likely to have come from different nests (Kaspari 1993; Brühl *et al*. 2003). A maximum of four workers per site (no more than one worker per quadrat) were selected for analysis, resulting in a total of 20 individuals analysed per 2-km transect in each forest location. Our sampling resulted in a total of 120 ant individuals being analysed.

Study sites

Study locations were in two areas in continuous lowland rainforest (Danum Valley Conservation Area, grid reference 4°55' N, 117°40' E; Maliau Basin Conservation Area, 4°49' N, 116°54' E) and one large forest fragment (Tabin Wildlife Reserve (WR), area = 111,971 ha; Figure 1). These three locations are termed "large" forests in subsequent analyses. These forest reserves are located in the Lahad Datu–Tawau district and surrounded by oil palm plantation, with Tabin WR surrounded by especially large tracts. Danum Valley and Maliau Basin are surrounded by secondary forests that were logged approximately 50 years ago. Samples were also obtained from three "small" forest fragments (Keruak Virgin Jungle Reserve (VJR; area = 225 ha), Pin Supu VJR (151 ha) and Sungai Sapi VJR (320 ha). These smaller fragments were protected Virgin Jungle Reserves which supported relatively undisturbed primary forest habitats, and thus were likely to contain forest of similar quality to the large forest sites. Keruak VJR and Pin Supu VJR are located in Kinabatangan District approximately 42–50 km from Sandakan. Both forest reserves are surrounded by oil palm plantation and small villages. The forest reserves have a mixture of swamp forest, limestone forest and dominantly mixed dipterocarp forest where timber was selectively extracted approximately 50 years ago. Sungai Sapi VJR is located along the Sandakan–Telupid highway: also surrounded by oil palm plantation. The forest is mainly lowland dipterocarp forest and partly heath forest. Secondary forest is commonly found adjacent to the oil palm plantations. These forests are suspected to have been isolated for approximately 40 years (McMurrow & Talip 2001). Distances between forests are provided in Table 2. Straight-line distances between locations were measured following the procedure in Benedick *et al*. (2007).

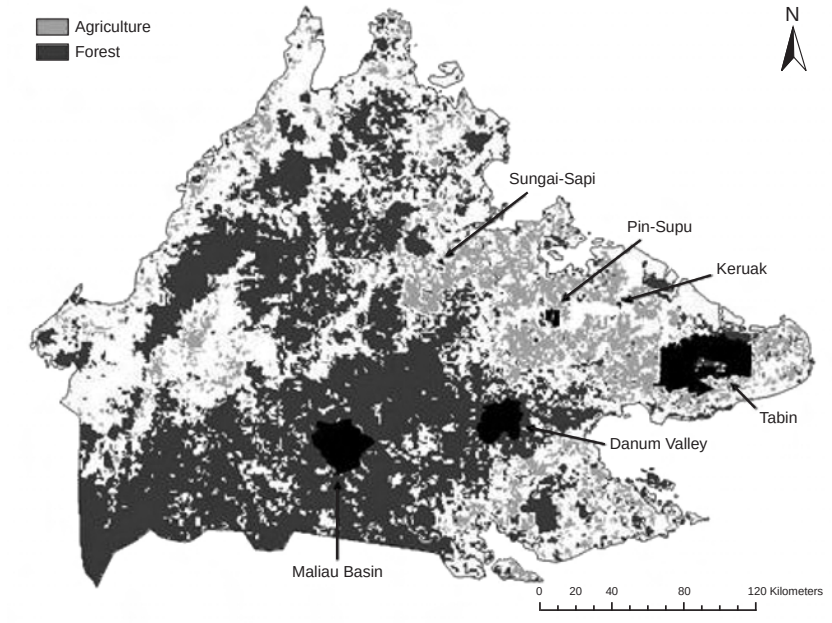

Fig. 1. Location of the study sites (black shading) in Sabah, Malaysian Borneo showing the large forest sites (Maliau Basin, Danum Valley and Tabin) and small forest remnants (Sungai Sapi VJR, Pin Supu VJR and Keruak VJR). Dark grey shading shows forest areas, light grey shading shows agricultural areas (i.e., oil palm plantations) and white shading shows unclassified land-uses (i.e., settlements, open lands, etc.). Source: World Database of Protected Areas; http://www.wdpa.org; Downloaded in 2009).

DNA tissue preparation and extraction

Each worker ant was placed in individual 1.5 ml centrifuge tubes with absolute ethanol stored at room temperature. Subsequently, the gaster was removed using a sterilized razor to reduce the amount of formic acid and other inhibitors, and DNA was extracted from head and thorax tissue. The tissue was transferred to new clean tubes with 100 µL extraction buffer containing 20 µL of proteinase K and RNAse A. The tissue was ground with a pestle and incubated at 55°C for 1 hour. Once the DNA was extracted, DNA samples were then eluted in Low TE buffer (40 µL of 10 mM Tris, 0.1 mM EDTA, pH 8.0) and stored at 4°C. Extracted DNA was purified using ethanol precipitation. DNA samples were mixed with 4 µL of 0.4 M sodium acetate and 100 µL of ice-cooled absolute ethanol, placed at minus 20°C for 1 hour and then centrifuged for 15 minutes at 15871 *g*. The samples were rinsed with 100 µL 70% ethanol for 5 minutes, centrifuged again at 15871 *g* for 15 minutes, and air-dried at room temperature. Finally, the dried DNA pellet was eluted with 20 µL TE and stored at minus 20°C prior to AFLP analysis.

AFLP analysis

AFLP analyses were conducted following protocols described by Whitlock *et al.* (2008a) with slight modifications to ensure that ant DNA inhibitors were reduced. Diluted DNA was subjected to restriction at 37°C for 3 hours using *EcoRI* and *MseI* endonucleases. Restricted DNA was ligated at 16°C overnight before diluting with 50 µL sterile water. Preselective PCRs were run using twice the volume of ligated DNA and PCR reaction mix than stated in Whitlock *et al.* (2008a): 10 µL total reaction volume. To ensure that the DNA was completely free from inhibitors during PCR reactions, the concentration of $MgCl_2$ was decreased (from 50 mM to 25 mM) and BSA (30 mg ml^{-1}) was added. Diluted pre-selective PCR product was subjected to selective PCR amplification by obtaining 1 µL of the diluted PCR products combined with PCR master mix. Three *EcoRI/MseI* primer combinations were used to generate the AFLP profiles (*EcoRI*-TCT/*MseI*-CGT; *EcoRI*-TCT/*MseI*-CGA; *EcoRI*-TCT/*MseI*-CTT). Selective PCR products were diluted by a factor of 1:100 and the samples were prepared for genotyping on the ABI 3730 sequencer.

Sequencing preparation protocol

Five µL of ABI Rox size standard was added to 1 ml HiDi formamide (ABI), and a 9.5 µL aliquot of this mix was added to each well of the sequencing plate with 0.5 µL of the diluted selective PCR products. The plate was denatured at 95°C for 3 minutes and placed on ice afterwards. The sequencing plate was sealed with a septum and then run on an ABI 3730 sequencer using the default settings for AFLP analysis.

Genotyping and AFLP scoring

GeneMapper software (version 3.7) was used to visualise the AFLP fingerprints; fragment size ranged from 50–500 base pairs (bp). GeneMapper was then used to assign bin positions and produce a matrix of peak heights for all the samples at these loci. The resulting peak height matrix was scored for presence or absence of bands using the "R" script AFLPscore version 1.3 (Whitlock *et al*. 2008b). This method removes the subjectivity associated with manually editing AFLP loci, whilst minimising error rates (Whitlock *et al*. 2008b). The binary genotype table indicating the presence or absence of a peak at each of the retained loci was then used to investigate genetic diversity and population structure.

Genetic diversity and population genetic structure

The proportion of polymorphic loci at the 95% level and the expected heterozygosity (Hj: which is analogous to Nei's gene diversity He) were determined using AFLP-SURV software (Vekemans *et al.* 2002). Genetic structure was determined using F_{ST} and AMOVA (Excoffier *et al.* 1992) using the Arlequin 3.11 software (Excoffier *et al*. 2005). Isolation-by-distance was examined using a Mantel test with AFLP-SURV version 1.0 software.

RESULTS

AFLP scoring

A total of 120 ant samples were analysed of which 110 samples produced useful data based on output generated from GeneMapper software. The ten samples that were discarded showed irregular profiles that could not be corrected. Table 1 shows the number of samples analysed from each of the six sample locations.

Genetic diversity and population genetic differentiation

A total of 168 loci were scored from six populations of *P. obscurans* based on AFLP analysis. There was little difference in the percentage of polymorphic loci recorded at locations following forest fragmentation (small fragments, mean = 79.2% (133/168 loci); large sites, mean = 75.0% (126/168 loci); Table 1). Of all six study locations, Sungai Sapi VJR (area 320 ha) had the highest percentage of polymorphic loci (89.3%) while Pin Supu VJR (151 ha) had the lowest (73.8%). Thus, there was little genetic variation observed overall, and the greatest variation was between two of the small forest sites. The mean expected heterozygosity was also similar between small forest fragments (Hj = 0.253; range 0.241–0.272) and large forest fragments (Hj = 0.246; range 0.193–0.276).

Populations of *P. obscurans* showed significant genetic differentiation based on one thousand random permutations of AFLP data (F_{ST} = 0.1614, df = 5, $P < 0.05$). There was significant genetic differentiation both among populations (F_{ST}; each forest fragment) and among populations within the two groups (F_{SC}; forests in large or small fragments), but not between the groups (F_{CT}; large and small fragments) based on a hierarchical AMOVA (AMOVA; F_{CT} = 0.03, df = 1, P = 0.31; F_{SC} = 0.15, df = 4, $P < 0.0001$; F_{ST} = 0.17, df = 104, $P < 0.0001$). Just 2.73 percent of the total variation observed was explained by fragment size. Pairwise, genetic differentiation was highest between a large site (Tabin Wildlife Reserve, area = 111,971 ha) and the smallest fragment (Pin Supu VJR, area = 151 ha; Slatkin's linearised F_{ST} = 0.7063, df = 5, $P < 0.05$; Table 2). The least-differentiated populations were in Danum Valley and Maliau Basin (Slatkin's linearised F_{ST} = 0.003, df = 5, $P > 0.05$), which were within the same large tract of continuous forest (Table 2). Sites separated by oil palm plantations were more genetically differentiated than the two locations linked by forest, and the smallest forest fragment (Pin Supu VJR, 151 ha, Table 2) was the most genetically differentiated of all the study sites. Overall, there was no significant relationship between geographical distance and genetic distance (pairwise F_{ST} values) between study locations (Mantel test; Z = 0.39, P = 0.08; N = 15), although the trend was approaching significance.

Table 1: AFLP data describing genetic diversity of *Pachycondyla obscurans* from six study locations, showing estimates of expected heterozygosity and the percentage of polymorphic loci

Population	Area (ha)	No. of samples	% polymorphic loci	Expected heterozygosity (Hj) ± S.E
Large forests				
Danum Valley	continuous	17	74.4	0.268±0.016
Maliau Basin	continuous	16	76.2	0.276±0.016
Tabin	111,971	20	74.4	0.193±0.013
Mean			75.0	0.246±0.015
Small forests				
Keruak VJR	225	19	75.0	0.241±0.015
Pin Supu VJR	151	19	73.8	0.245±0.015
Sungai Sapi VJR	320	19	89.3	0.272±0.012
Mean			79.4	0.253±0.014

Table 2: Pairwise genetic distances between study populations estimated by Slatkin's linearized genetic distance (upper triangle). Shortest straight-line distances between boundaries of forest study locations (in km) are also shown (lower triangle)

Site	Maliau	Danum	Tabin	Sungai Sapi	Pin Supu	Keruak
Maliau	-	0.0003	0.1500	0.0892	0.2969	0.0364
Danum	59	-	0.1327	0.0655	0.3245	0.0255
Tabin	164	82	-	0.1497	0.7063	0.1817
Sungai Sapi	102	85	132	-	0.3695	0.0240
Pin Supu	114	50	59	64	-	0.3587
Keruak	155	81	35	102	35	-

DISCUSSION

Genetic diversity

There were no differences in genetic diversity of *P. obscurans* according to fragment size (large versus small locations), contrary to a previous study of ants which reported reduced genetic diversity in fragments (Bickel *et al*. 2006). However, in that previous study, significantly reduced diversity in small fragments was observed in only one ant species (*Pheidole annexus*; Bickel *et al*. 2006), with no differences recorded in another ant species (*Odontomachus rixosus*), implying that genetic effects of fragmentation vary among ant species. In addition, a study of butterflies in Sabah also found little effect of forest fragmentation on genetic diversity (Benedick *et al*. 2007). Benedick *et al*. (2007) sampled in five of the six sites sampled in this study (all but Pin Supu VJR), and the similar results in the two studies imply that fragmentation had relatively little impact on genetic diversity in either of these two insect taxa. In both these studies, the smallest fragments surveyed were more than 100 ha, and the relatively large size of the smallest forest remnant sampled may help explain the lack of any significant changes in genetic diversity in either ants or butterflies. Many other studies of habitat fragmentation sampled much smaller fragments (e.g., 20 ha fragment; Bickel *et al*. 2006) than considered here, and this may account for the reduced genetic diversity they reported. As pointed out by Benedick *et al*. (2007), even though many butterfly species fail to disperse through agricultural areas, the relatively short time since fragmentation in Sabah (~50 years) and the relatively large population sizes of butterflies in fragments may also contribute to the lack of any genetic erosion being evident within butterfly populations. We have no information on the population sizes of *P. obscurans* in our study sites, but the fact that the species was commonly recorded at sites during our study implies that the effective population size may be relatively large. There may also be a time lag between fragmentation and genetic erosion such that longer periods of time (and more insect generations) are required before effects of fragmentation are manifested.

The results from this study are likely to be robust and unlikely to be due to a sampling artefact because similar numbers of ants were sampled from each population. In addition, distances between sites along transects mean that samples were likely to be of individuals from different colonies. Nonetheless, only six forest locations were compared and so the analysis may have lacked power in this respect. The lack of any decline in genetic diversity in small fragments was supported by the similar proportion of polymorphic loci at all study locations (Table 1), also implying little effect of fragment size on ants. Thus, we conclude that the populations within the study locations were sufficiently large and/or fragmentation had occurred sufficiently recently that few differences in genetic diversity were present in relation to fragment size. The dispersal ability of *P. obscurans* is not known and there is no quantitative information on its ability to persist in disturbed habitats. Nonetheless, it is possible that lack of changes in genetic diversity may also be due to sufficient gene flow between sites if reproductive adults can fly between

fragments across the intervening oil palm habitat. Further studies are required for sampling ants within agricultural landscapes to investigate this possibility. However, the lack of any genetic changes in diversity suggests that the smallest forest fragments sampled in this study currently support genetically viable populations of the ant *P. obscurans*.

Some studies have hypothesized that genetic differentiation of invertebrates in fragmented forests occurs after approximately 200 years (Keller & Lagiarder 2003; Keller *et al*. 2004), and Benedick *et al*. (2007) suggested that at least one hundred generations (years) may be necessary for genetic erosion effects to be evident in butterflies. Time periods taken to observe genetic erosion in ants are likely to be even greater than in butterflies because queens can live up to 40 years (Hölldobler & Wilson 1990). Moreover, because ants live in colonies where only queens and males reproduce sexually, the effective population size can be small relative to nest size. Thus, the length of time for genetic erosion to occur may be much longer than the time-since-fragmentation in this study and may explain why few differences were observed. Nonetheless, Bickel *et al*. (2006) showed that nucleotide diversity of the generalist ant (*Pheidole annexus*) was lower in forest fragments in the same study region (Sabah). This difference between species may have been due to differences in their ecology, such as nesting preferences, colony size, and body size (Bickel *et al*. 2006). Differences in sizes of fragments sampled, or in the degree of habitat disturbance within fragments or the nature of the surrounding matrix (oil palm plantation), may also account for these different results, as well as differences in species dispersal ability and habitat associations, and more studies are required.

Population genetic differentiation

The lack of any isolation-by-distance effect in this study may reflect that approximately 50 years of isolation may not be sufficient to affect dispersal and gene flow among fragments. *Pachycondyla obscurans* is a forest specialist and has not been recorded in oil palm plantations, and so this species is unlikely to be able to disperse across agricultural landscapes and genetic differences among fragments may emerge over time assuming that winged adults cannot disperse between fragments. All locations separated by oil palm plantations were more genetically differentiated than were populations from two locations in continuous forest, supporting the notion that oil palm habitats prevent barriers to dispersal. These locations in continuous forest were not in particularly close proximity (~60 km apart) but are linked by forest habitat which may be important for maintaining gene flow and reducing threats from genetic erosion.

Conclusion

To our knowledge, this is the first report on the use of AFLP techniques to study genetic diversity of ants in fragmented tropical forests. Similar studies by Bickel *et al*. (2006) used Randomised Amplified Polymorphic DNA (RAPD). RAPDs are considered to be less powerful than AFLPs for studying intra- and inter-specific differentiation (Gyzl *et al*. 2004), implying that our methods would have been more likely to detect an effect of fragmentation if one had been present.

Our study showed that genetic diversity of a ground-dwelling ant was not greatly affected by forest fragment size, and approximately 40 years since fragmentation may not be a sufficiently long time to detect any isolation-by-distance effects. Our findings suggest that forest fragments larger than 150 ha may be of especially high conservation value and may support viable populations of ants. Two study locations that were in the same block of continuous forest were most genetically similar, even though these sites were 60 km apart. By contrast, ant populations in sites separated by oil palm plantations were more genetically distinctive, indicating that the nature of the intervening matrix is important. There is an urgent need to evaluate the conservation value of much smaller forest fragments in Sabah, and to examine the degree to which forest species can disperse across agricultural landscapes, such as oil palm plantations. Improving landscape connectivity in agricultural landscapes may help promote dispersal of forest species and gene flow among forest fragments.

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REFERENCES

Benedick S, Hill JK, Mustaffa N, Chey VK, Maryati M, Searle JB, Schilthuizen M and Hamer KC, 2006. Impacts of rain forest fragmentation on butterflies in northern Borneo: Species richness, turnover and the value of small fragments. *Journal of Applied Ecology* 43(5): 967–977.

Benedick S, White TA, Searle JB, Hamer KC, Mustaffa N, Chey VK, Maryati M, Schilthuizen M and Hill JK, 2007. Impacts of habitat fragmentation on genetic diversity in a tropical forest butterfly on Borneo. *Journal of Tropical Ecology* 23(6): 623–634.

Bickel TO, Brühl CA, Gadau JR, Hölldobler B and Linsenmair E, 2006. Influence of habitat fragmentation on the genetic variability in leaf litter ant populations in tropical rainforests of Sabah, Borneo. *Biodiversity and Conservation* 15(1): 157–175.

Boswell GP, Britton FN and Franks NR, 1998. Habitat fragmentation, percolation theory and the conservation of a keystone species. *Proceedings of the Royal Society of London Series B Biological Sciences* 265: 1921–1925.

Brook BW, Tonkyn DW and Frankham R, 2002. Contribution of inbreeding to extinction risk of threatened species. *Conservation Ecology* 6(1): 16.

Brown KS and Hutchings RW, 1997. Disturbance, fragmentation and the dynamics of diversity in Amazonia forest butterflies. In: *Tropical Forest Remnants* (Laurance RW), University of Chicago Press, Chicago, Illinois, 91–110.

Brühl CA, Eltz T and Linsenmair E, 2003. Size does matter—effects of tropical rainforest fragmentation on the leaf litter ant community in Sabah, Malaysia. *Biodiversity and Conservation* 12(7): 1371–1389.

Brühl CA and Eltz T, 2010. Fuelling the crisis: species loss of ground-dwelling forest ants in oil palm plantations in Sabah, Malaysia (Borneo). *Biodiversity & Conservation*. 19(2):519–529.

Davies KF and Margules CR, 1998. Effects of habitat fragmentation on carabid beetles: experimental evidence. *Journal of Animal Ecology* 67(3): 460–471.

De Souza OFF and Brown VK, 1994. Effects of habitat fragmentation on Amazonia termite communities. *Journal of Tropical Ecology* 10(2): 85–98.

Didham RK, 1997. The influence of edge effects and forest fragmentation on leaf litter invertebrates in Central Amazonia. In: *Tropical Forest Remnants*: *Ecology, Management and Conservation of Fragmented Communities* (Laurance RW), University of Chicago Press, Chicago, Illinois, 55–70.

Didham RK, Ghazoul J and Stork NE, 1996. Insects in fragmented forest: a functional approach. *Trends in Ecology and Evolution* 11(6): 255–260.

Excoffier L, Laval G and Schneider S, 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1:47–50.

Excoffier L, Smouse PE and Quattro JM, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes—application to human mitochondrial DNA restriction data. *Genetics* 131(2): 479–491.

Frankham R, 1998. Inbreeding and extinction: island population. *Conservation Biology* 12:665–675.

Gyzl A, Augustynowicz E, Mosiej E, Zawadka M, Guiadek G, Nowaczek A and Slusarczyk J, 2004. Amplified fragment length polymorphism (AFLP) versus randomly amplified polymorphism DNA (RAPD) as new tool for inter- and intra-specific differentiation with *Bordetella*. *Journal of Medical Microbiology* 54(4): 333–346.

Hamer KC, Hill JK, Benedick S, Mustaffa N, Sherratt TN, Maryati M and Chey VK, 2003. Ecology of butterflies in natural and selectively logged forest of northern Borneo: the importance of habitat heterogeneity. *Journal of Applied Ecology* 40(1): 150–162.

Hartl D and Clark AG, 1989. *Principles of Population Genetics*. 2nd edition. Sinauer Associates Inc. Publishers, Sunderland Massachusetts.

Hölldobler B and Wilson EO, 1990. *The ants*. Harvard University Press, Cambridge, Massachusetts, USA.

Kaspari M, 1993. Body-size and microclimate use in neotropical granivorous ants. *Oecologia* 96(4): 500–507.

Keller I and Largiader CR, 2003. Five microsatellite DNA markers for the ground beetle *Abax parallelepipedus* (Coleoptera, Carabidae). *Molecular Ecology* 3(1): 279–293.

Keller I, Nentwig W and Largiader CR, 2004. Recent habitat fragmentation due to roads can lead to significant genetic differentiation in an abundant flightless ground beetle. *Molecular Ecology* 13(10): 2983–2994.

Kitching RL, Orr AG, Thalib L, Mitchell H, Hopkins MS and Graham AW, 2000. Moth assemblages as indicators of environmental quality in remnants of upland Australian rain forest. *Journal of Applied Ecology* 37(2): 284–297.

Klein BB, 1989. Effects of forest fragmentation on dung and carrion beetle communities in central Amazonia. *Ecology* 70(60): 1715–1725.

Laurance WF, Vasconselos HL and Lovejoy TE, 2000. Forest loss and fragmentation in the Amazon: Implications for wildlife conservation. *Oryx* 34(1): 39–45.

Luikart G, England PR, Tallmon D, Jordan S and Taberlet P, 2003. The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics* 4: 981–994.

Lynch M, 2002. Gene duplication and evolution. *Science* 297:945–947.

MacArthur RH and Wilson EO, 1967. *The theory of island of biogeography*. Princeton University Press, New Jersey.

McMorrow J and Talip MA, 2001. Decline of forest area in Sabah, Malaysia: Relationship to state plocies, land code and land capability. *Global Environmental Change-Human and Policy Dimensions* 11:217–230.

Monaghan MT, Spaak P and Robinson CT, 2002. Population genetic structure of 3 alpine stream insects: influences of gene flow, demographics and habitat fragmentation. *Journal of the North American Benthological Society* 21(1): 114–131.

Myers N, 2003. Biodiversity hotspots revisited. *Bioscience* 53(10): 916–917.

Peters MK and Okalo B 2009. Severe declines of ant-following birds in African rainforest fragments are facilitated by a subtle change in army ant communities. *Biological Conservation* 142(10): 2050–2058.

Ravel S, Monteny N, Olmos DV, Verdugo JE, and Cuny G, 2001. A preliminary study of the population genetics of *Aedes aegypti* (Diptera: Culicidae) from Mexico using microsatellite and AFLP markers. *ACTA Tropica* 78(3): 241–250.

Rosenzweig ML and Clark CW, 1995. Island extinction rates from regular consensus. In: *Readings from Conservation Biology: Genes, population and species* (Ehrenfeld D), Blackwell Scientific Publication, Massachusetts, USA, 112–115.

Schoereder JH, Sorbrino TG, Ribas CR and Campos RBF, 2004. Colonization and extinction of ant communities in a fragmented landscape. *Austral Ecology* 29(4): 391–398.

Stanton S, Honnay O, Jacquemyn H and Roldan-Ruiz I, 2009. A comparison of the population genetic structure of parasitic *Viscum album* from two landscapes differing in degree of fragmentation. *Plant Systematics and Evolution* 281(1–4): 161–169.

Templeton AR, Robertson RJ, Brisson J and Strasburg J, 2001. Disrupting evolutionary processes: the effect of habitat fragmentation on collard lizards in the Missouri Ozarks. *Proceedings of the National Academy of Sciences USA* 98:5426–5432.

Turner IM, 1996. Species loss in fragments of tropical rain forest: a review of the evidence. *Journal of Applied Ecology* 33:200–209.

Vasconselos HL, Vilhena JMS, Magnusson WE and Albernaz ALKM, 2006. Long-term effects of forest fragmentation on Amazonian communities. *Journal of Biogeography* 33:1348–1356.

Vekemans X, Beauwens T, Lemaire M and Roldan-Ruiz I, 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology* 11:139–151.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M and Zabeau M, 1995. AFLP—a new technique for DNA fingerprinting. *Nucleic Acids Research* 23:4407–4414.

Whitlock R, Hipperson H, Mannarelli M and Burke T, 2008a. A high-throughput protocol for extracting high-purity genomic DNA from plants and animals. *Molecular Ecology Resources* 8:736–741.

Whitlock R, Hipperson H, Mannarelli M, Butlin RK and Burke T, 2008b. An objective, rapid and reproducible method for scoring AFLP peak-height data that minimizes genotyping error. *Molecular Ecology* 8(4): 725–735.

Yamane S, 2007. *Pachycondyla nigrita* and related species in Southeast Asia. In: Advances in ant systematics (Hymenoptera: Formicidae): homage to E.O. Wilson–50 years of contribution (Snelling RR, Fisher BL and Ward PS, eds), *Memoir of the American Entomology Institute* 80:650–663.