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Short-Term Impact of 1997/1998 ENSO-Induced Disturbance on Abundance and Genetic Variation in a Tropical Butterfly

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Abstract

In order to assess the short-term impact of habitat loss after disturbance, we studied *Arhopala epimuta* (Lepidoptera: Lycaenidae) populations in 5 landscapes in Borneo that were differentially affected by the 1997/1998 El Niño Southern Oscillation–induced drought and fire. Sampling was conducted before (1997) and after (1998 and 2000) disturbance. This study combined demographic and genetic data inferred from the analysis of 5 microsatellite loci and mitochondrial DNA (mtDNA) control region sequences. Over all 5 landscapes, a total of 313 *A. epimuta* were sampled over the 3-year survey. Butterfly abundance varied greatly both spatially and temporally (within disturbed landscapes). After the disturbance, a 4-fold population expansion was observed in a small unburned isolate, whereas population extinction was observed in one of the severely burned areas. The analysis of mtDNA sequences in a subsample of 106 *A. epimuta* revealed no significant spatial or temporal genetic structure. The analysis of 5 microsatellite loci revealed high frequencies of null alleles. Genetic evidence of recent change in population size was found in all 3 unburned landscapes using microsatellites. Congruent to mtDNA, microsatellites failed to detect significant genetic structure according to sampling year or landscapes. Our results suggest that, for mobile species within recently fragmented habitat, habitat loss after disturbance may lead to local population extinction but may augment genetic diversity in remnant local populations because of increased gene flow.

The effect of habitat disturbance on genetic and demographic viability of species has become an important issue over recent decades (Wilcox and Murphy 1985; Saunders and others 1991). The fragmentation of continuous suitable habitat is usually related to disturbance and leads to patchily distributed populations that can be both demographically and genetically modified (Saccheri and others 1998). The extent to which a particular species will be affected depends on the magnitude of the disturbance, the degree of habitat specialization of the species, and its dispersal potential and behavioral response to habitat fragmentation (Weins 1997). For instance, strongly dispersing generalist species may actively move among patchy habitats, increasing local genetic diversity and maintaining gene flow among isolated locations (Porter 1999). In contrast, decreased habitat patch connectivity will be observed in species with limited vagility, leading

to increases in genetic divergence between remnant populations (Harrison and Hastings 1996). Furthermore, habitat losses are expected to result in population size reductions that may in turn increase inbreeding rates, losses of genetic variation, and fixation of deleterious mutations, further reducing the evolutionary potential of remnant isolated populations and increasing their risk of extinction (Chakraborty and Nei 1976; Frankham 1995; England and others 2003). After these bottlenecks, population recoveries (both in size and in genetic diversity) will depend on species turnover, number of remaining breeders, stochasticity of reproductive success, species dispersal abilities, and the geographic scale at which the disturbance occurred.

El Niño Southern Oscillation (ENSO)–induced droughts and fires represent a major disturbance and cause of habitat loss and fragmentation in tropical rain forests. The Indonesian

province of East Kalimantan (Borneo) was severely affected by the 1997/1998 ENSO event. More than 5 million ha burned in East Kalimantan alone, making it by far the most severe and widespread event recorded in Borneo (Harrison 2000; Siegert and others 2001). Human-induced forest fires associated with ENSO-related droughts had a profound impact on ecosystem dynamics, community composition, and species richness (Holmgren and others 2001; Cleary 2003; Laurance 2003).

Butterflies in Borneo have previously been extensively studied in terms of community responses to both logging and ENSO-induced droughts and fires (Willott and others 2000; Cleary 2003; Hamer and others 2003; Cleary and Genner 2004; Cleary and Mooers 2004; Cleary and others 2004). In East Kalimantan, the fires created a mosaic of unburned forests (some of which were affected by drought), isolating local butterfly habitats and reducing species richness in the affected areas (Cleary 2003; Cleary and Genner 2004). Butterflies are a particularly good model for studying the genetic effects of disturbance and habitat loss because landscape composition is an important determinant of butterfly population structure (Brookes and others 1997; Lewis and others 1997; Saccheri and others 1998; Keyghobadi and others 1999; Schmitt and Seitz 2002; Harper and others 2003; Williams and others 2003).

Here, we studied the effects of disturbance on abundance and population genetic structure in a tropical butterfly, *Arhopala epimuta* (Lepidoptera, Lycaenidae). *Arhopala epimuta* is a common forest-dependent lycaenid found in Borneo. Lycaenids represent more than 47% of butterfly (Papilionoidea) species (Robins 1982), and the *Arhopala* group of species is extraordinarily diverse, with more than 200 described species (Bridges 1988; D'Abbrera 1990) of which 88 are found in Borneo (Seki and others 1991). Little is known, however, about genetic variation within species, and this study is the first to attempt a detailed analysis of this diversity. *Arhopala* species are usually found in pristine forest and are presumably moderate to poor dispersers as are most lycaenids (Dennis 1992). The larvae only feed on tree species, with some species-specific variation in diet (some are narrowly oligophagous, others polyphagous). In addition, lycaenid larvae including *Arhopala* often display a variety of interactions with ants (Fiedler 1991; Pierce and others 2002). Little is known about host plant and ant association in *A. epimuta*. However, according to Megens and others (2005), *A. epimuta* feeds on young foliage of Fagaceae (beech family), as do most closely related species. Thus, *A. epimuta* can be classified as a specialist in terms of its larval host and obligate myrmecophily (i.e., larvae are associated with ants), even though *Arhopala atosia* (the closest related species) has been associated with at least 3 ant species (Megens and others 2005).

The main goal of our study was to assess the change in *A. epimuta* abundance and genetic makeup relative to the 1997/1998 ENSO-induced disturbance. We therefore assessed *A. epimuta* abundance and genetic composition through 3 years of surveys (1997, 1998, and 2000) in 5 different landscapes differentially affected by the 1997/1998 ENSO-induced drought and fire. A standardized method was used to esti-

mate abundance within landscapes, and the genetic composition was assessed using 2 types of markers: sequences of the maternally inherited mitochondrial DNA (mtDNA) and 5 microsatellite loci.

Materials and Methods

Study Area

Butterfly surveys were conducted in 1997 (before the fires), 1998, and 2000 in the Balikpapan–Samarinda region of East Kalimantan, Indonesian Borneo (Cleary 2003; Cleary and Genner 2004) (Figure 1). During these surveys, 5 differentially disturbed landscapes from 3 regions were sampled. Landscapes in unburned isolates (I1, I2, and I3) and burned forests (B1 and B2) were located in the 5.2 million ha of East Kalimantan that changed from a habitat mosaic of forest with areas of secondary growth to an area dominated by secondary (burned) forest with only remnant unburned patches (Siegert and others 2001). All landscapes are fully described in terms of exact location and dominant vegetation in Cleary (2003). Briefly, I1 (unlogged primary) and I3 (logged in 1993/1994) landscapes were located in a 108 000-ha isolate within the so-called Gunung Meratus region, which includes a part of the International Timber Concessions Indonesia and adjacent Balikpapan Forest Industries concessions and the Gunung Meratus Protected Forest Reserve (ca. 30 000 ha) (Figure 1, Table 1). Within the so-called Sungai Wain region, I2 landscape was located in a 3500-ha unburned primary isolate that is all that remains of the Sungai Wain Protection Forest (6500 ha of the original 10 000 ha burned during the 1997/1998 ENSO event); the burned landscape B1 was located in the burned part of the nature reserve. The second burned landscape B2 is located in the Wanariset Samboja Research Forest that partially burned during the 1982/1983 ENSO event and severely burned during the 1997/1998 ENSO event. None of the burned landscapes have been commercially logged, although they may have been subjected to unrecorded illegal logging.

Sampling, Preservation, and DNA Extraction

Specimens of *A. epimuta* were sampled along 8–16 transects of 300 × 30 m each within each landscape, as described in Cleary (2003). Along transects, the number of observed *A. epimuta* was first recorded prior to sampling. Abundance within transects was estimated as the number of recorded individuals per transect per day in order to correct for variations in the time used to sample transects. Abundance within landscapes was estimated as the average abundance observed within sampled transects. Collected individuals were immediately killed after which 3 legs were removed and stored in 96% ethanol. These voucher specimens were preserved (using silica gel) and have been deposited in the Zoological Museum of the University of Amsterdam, the Netherlands. Genomic DNA was isolated from one ground

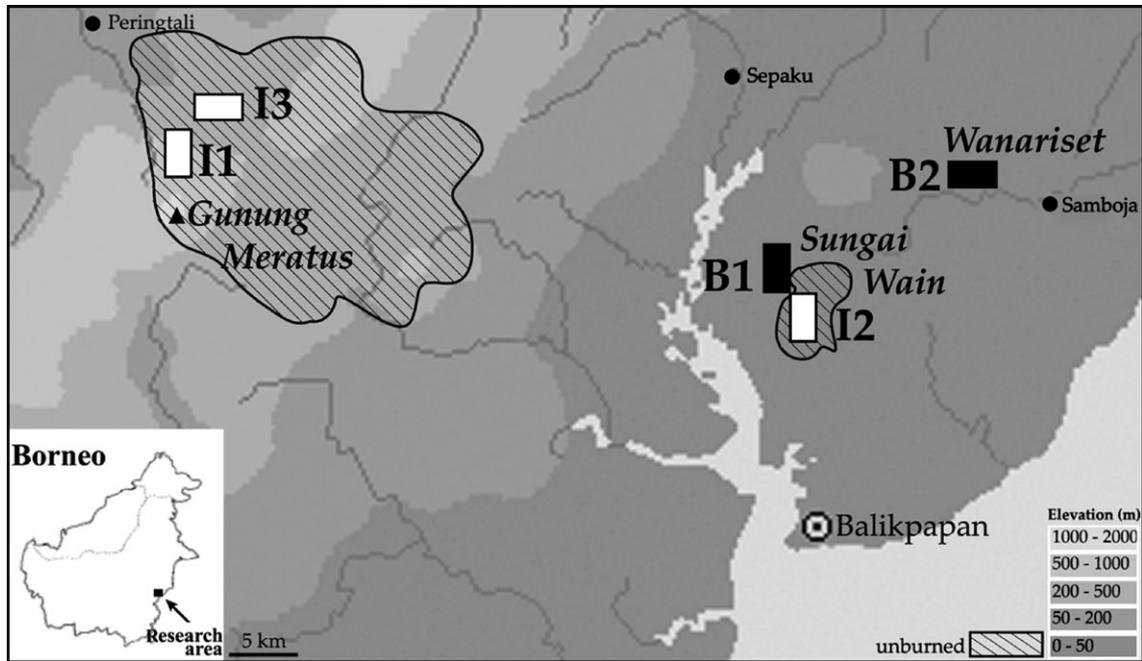


Figure 1. Map of the research area, located in the Balikpapan–Samarinda region of East Kalimantan (Indonesian Borneo), showing the location of landscapes in which individuals of *Arhopala epimuta* were sampled. Black rectangles represent burned landscapes, and white rectangles represent unburned isolates. I1 and I3 landscapes were located in the so-called Gunung Meratus region and were sampled in 2000. B1 and I2 landscapes were located in the Sungai Wain Protection Forest and were sampled in 1998 and in 2000. B2 was located in the Wanariset forest and was sampled in 1997, 1998, 1999, and 2000.

leg using a cetyltrimethyl ammonium bromide protocol (Hillis and Moritz 1990).

The mtDNA Sequencing and Data Analysis

Amplifications of the mtDNA control region (CR) plus a fragment of the 12S rDNA gene were conducted using primers SeqLepMet and LepAT2B (Vila and Björklund

2004). Polymerase chain reaction (PCR) was performed in 25 μ l containing 1 \times PCR buffer (HT Biotechnology, Cambridge, UK), 3 mM MgCl₂, 0.12 mM of each deoxynucleoside triphosphate, 0.5 mg/ml bovine serum albumin, 0.05 μ M of each primer, 0.4 U *Taq* polymerase (HT Biotechnology), and approximately 20 ng DNA. PCRs were carried out in PTC 100 Thermocycler (MJ Research, Waltham, MA): 2 min

Table 1. Sampling information, number of recorded and analyzed *Arhopala epimuta* (numbers in parentheses represent numbers of individuals for which the mtDNA CR was sequenced), and estimated abundance (average number of individuals per transect per day) within each sampled landscape. Samples are named according to the sampled landscape (see Figure 1) and the year of sampling. The forest status refers to the degree of disturbance of the landscape at the time of sampling. For example, B1 was originally a pristine forest that burned during the 1997/98 ENSO event

Sample name	Sampled forest	Sampled year	Forest status	No. sampled transects	Recorded individuals	Estimated abundance	Analyzed individuals
I1-00	Gunung Meratus	2000	Pristine	16	57	0.52	48 (22)
I3-00	Gunung Meratus	2000	Logged 1993/1994	16	140	1.49	77 (22)
B1-98	Sungai Wain	1998	Burned 1997/1998	16	7	0.14	4 (4)
B1-00	Sungai Wain	2000	Burned 1997/1998	16	33	0.38	6 (6)
I2-98	Sungai Wain	1998	Pristine dry 1997/1998	16	27	0.60	18 (15)
I2-00	Sungai Wain	2000	Pristine dry 1997/1998	16	259	2.51	110 (20)
B2-97	Wanariset	1997	Burned 1982/1983	8	64	1.31	48 (17)
B2-98	Wanariset	1998	Burned 1997/1998	9	0	0	0
B2-00	Wanariset	2000	Burned 1997/1998	9	0	0	0

denaturation at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 54 °C, and 1 min at 65 °C; and a final extension of 5 min at 65 °C. Amplified fragments were purified with the BOOM procedure (Boom and others 1990) and cycle sequenced with either SeqLepMet or LepAT2B using Big Dye Terminator sequencing kit (Applied Biosystems, Foster City, CA). Sequences were run on an ABI 3100 Genetic Analyzer (Applied Biosystems). Forward and reverse sequences were compared for each individual in BIOEDIT (Hall 1999). Individual sequences were then aligned by eye in GENEDOC (Nicholas KB and Nicholas HB 1997). For further analysis, indels were coded as substitutions, and dinucleotide indels situated in the [TA]_n-like stretch were coded as single-base substitutions (see Results).

Haplotypes were identified, and their relative frequency within populations was calculated using ARLEQUIN version 2.000 (Schneider and others 2000). A minimum spanning network displaying evolutionary relationships between haplotypes was obtained in ARLEQUIN and drawn by hand in Adobe Illustrator 9.0 (Adobe Systems Incorporated, San Jose, CA).

Genetic diversity within the population was measured as the haplotype diversity (h , Nei 1987), the nucleotide diversity (π , Nei 1987), and the mean number of pairwise nucleotide differences between individuals (k , Tajima 1983) using DNASP 4.0 (Rozas and others 2003). Tajima's D -test (Tajima 1989) as well as the haplotype diversity test (h -test; Depaulis and Veuille 1998) was conducted within each sample using DNASP 4.0. For both tests, computer simulations were based on the coalescent process for a neutral infinite-sites model under the assumption of a large constant population size (Hudson 1990). DNASP generates the empirical distribution of D and h , obtained from 10 000 simulations under the neutral coalescent process and given the number of segregating sites observed in each sample (the model therefore considers that the number of segregating sites is fixed and that mutations are uniformly and randomly distributed along lineages). DNASP thus estimates the probability of obtaining lower or higher values of these parameters computed from the simulation than the one observed in each sample.

Data analyses of spatial and temporal genetic structure among populations were performed using ARLEQUIN. Genetic divergences between pairwise samples were estimated using Φ -statistics (Φ_{ST} based on haplotype frequencies and molecular divergence using pairwise differences). P values were obtained using a nonparametric permutation procedure with 10 000 permutations. The distribution of genetic variation was examined using an analysis of molecular variance (AMOVA; Excoffier and others 1992) to test the null hypothesis that genetic variation was not associated with temporal variation within landscapes or with spatial structure among regions (i.e., Gunung Meratus, Sungai Wain, and Wanariset).

Microsatellite Genotyping and Data Analysis

Genotypes at 5 microsatellite loci recently isolated for *A. epimuta* (Fauvelot 2005) were amplified according to the described

protocol. PCR products were diluted 20 times, and run on a Li-Cor 4200 automatic sequencer. To ensure scoring accuracy and consistency across gels, a locus-specific size standard from individuals with known genotypes was run at both sides of each gel, as well as a commercial size standard (50–350 bp, Li-Cor, Lincoln, NE). Individuals for which no PCR products were visualized on gel were reamplified during a new PCR using a 2 degrees lower annealing temperature.

Genetic diversity within samples was estimated as the observed heterozygosity (H_O) and the mean number of alleles per locus in GENETIX (Belkhir and others 2002), as well as the allelic richness (El Mousadik and Petit 1996) and the unbiased gene diversity (H_N , Nei 1987) in FSTAT version 2.9.3.2 (Goudet 2001). Deviations from Hardy–Weinberg (HW) equilibrium were estimated using the inbreeding coefficient f of Weir and Cockerham (1984) (equivalent to Wright's F_{IS}), and departures from HW expectations were assessed using exact tests with a Markov chain method in GENEPOP version 3.4 (Raymond and Rousset 1995). Linkage disequilibrium between all pairwise locus combinations was tested using the exact probability test in GENEPOP.

Null alleles were detected with MICRO-CHECKER (van Oosterhout and others 2004). When a significant heterozygosity deficiency was observed, null allele frequencies were estimated following Brookfield's (1996) null allele estimator 2, and the genotypes were adjusted by 1) estimating the number of true homozygotes according to HW expectations and 2) replacing the "false" homozygotes by genotypes made of one known allele and one missing allele. Even though adjusted data sets no longer contain multilocus genotypes, allelic frequencies can still be used for population genetic analyses, assuming that loci with null alleles are unlinked to other loci (van Oosterhout and others 2004). This corrected data set was therefore used to reestimate the inbreeding coefficient f of Weir and Cockerham (1984) and assess locus conformance to HW expectations as described previously.

To test for evidence of a recent reduction in effective population size, the adjusted microsatellite data set was analyzed using BOTTLENECK 1.2.02 (Cornuet and Luikart 1996; Piry and others 1999). This test detects significant differences between the gene diversity (H_{Exp}) and the expected equilibrium heterozygosity (H_{Eq}) calculated through simulations from the observed number of alleles at each locus, under various mutation models (Luikart and Cornuet 1998). A significant excess or deficiency of H_{Exp} compared with the H_{Eq} can be interpreted as a signature of a recent change in population size (Luikart and Cornuet 1998). H_{Eq} was calculated under the stepwise mutation model (SMM) and the 2-phased mutation model (TPM) with 95% single-step mutations. Significant differences between H_{Exp} and H_{Eq} were detected using the Wilcoxon signed-rank test.

Pairwise genetic divergences were estimated from the noncorrected data set using multilocus the estimators of F_{ST} of Weir and Cockerham (1984), and their significance was tested with 10 000 permutations of individuals among samples in GENETIX. As the adjusted data set no longer contained multilocus genotypes, we could only perform

single-locus AMOVAs using microsatellite allele frequency including null alleles, as described in Keyghobadi and others (2005).

Results

Arhopala epimuta Abundance

Over all 5 landscapes, a total of 587 *A. epimuta* were recorded during the 3 years survey (Table 1). Abundance varied greatly between landscapes at a set time and between years in specific landscapes. For example, in the Sungai Wain Protection Forest, abundance were estimated as 0.38 and 2.51 individuals per transect per day in 2000 in proximate B1 and I2, respectively, and in this same forest, within the landscape I2, estimations were 0.6 and 2.51 individuals per transect per day in 1998 and 2000, respectively. Within landscapes, there were large variations in *A. epimuta* abundance among the sampled transects, with the exception of the unburned landscape I2 from which the number of recorded individuals was similar between all 16 sampled transects (data not shown). No *A. epimuta* were recorded after the 1997/1998 ENSO event in the severely burned landscape B2 in Wanariset forest, despite relatively high butterfly abundance in 1997 (1.3 individuals per transect per day) and similar surveys conducted in 1998, 1999, and 2000 (including a substantial increase in sampling effort in 2000). A total of 313 *A. epimuta* were collected over the 5 landscapes and 3 years in order to conduct the genetic analysis.

Genetic Diversity and Neutrality Tests—mtDNA

A subsample of 106 *A. epimuta* was randomly selected to conduct mtDNA sequence analysis. The amplified mtDNA fragment, from which 463–467 continuous nucleotides could be reliably read for all specimens, contained the CR (327–331 bp) plus a fragment of 136 bp of the 5'-end of the 12S rDNA gene. Sequence length variations observed among individuals within the CR originated from length polymorphism within region D (the [TA]_n-like stretch) and the homopolymer situated at the 3'-end of the CR described in Vila and Björklund (2004). Because length polymorphism observed in region D originated from dinucleotide [TA] indels (bp 68 and 69, Table 2), each of these dinucleotide indels were treated as a single-base substitution. Among the 106 individuals of *A. epimuta* sequenced, we identified 37 haplotypes distinguished by 29 polymorphic sites, from which 14 were singletons (Table 2).

Among haplotypes, the uncorrected sequence divergence ranged from 0.21% to 1.50%. Overall, haplotype diversity (h) was 0.861, nucleotide diversity (π) was 0.00391, and the mean number of pairwise differences (k) was 1.821.

The minimum spanning network (Figure 2) presents the relationships among the 37 mtDNA haplotypes. Haplotypes H1 and H2 (differentiated by one transition) were the most common, representing 32% and 17.9% of overall samples, respectively (Table 3). A total of 7 haplotypes were shared by at least 2 landscapes. All other 30 haplotypes were shared

by a maximum of 2 individuals restricted to a single landscape. Within samples, the genetic diversity varied widely both spatially and temporally (Table 3). Tajima's D -tests did not detect deviations from neutral expectations in any of the landscapes (all $P > 0.05$), but the overall sample significantly deviated from neutral expectations ($P = 0.006$). The observed haplotype diversity within I2-98 was significantly higher than expected under the neutral coalescent process (Table 3; b -test, I2-98, average $b_{exp} = 0.704$; $P > 0.999$).

Genetic Diversity and HW equilibrium—Microsatellites

Among 313 individuals analyzed at 5 microsatellite loci, the number of alleles per locus varied from 13 (in *AeF10*) to 32 (in *AeB9*). Over all loci, the expected heterozygosity ranged from 0.690 (in B1-00) to 0.778 (in B1-98), whereas the observed heterozygosity ranged from 0.362 (in I3-00) to 0.441 (in I2-98) (Table 4). Allelic richness, based on a minimum sample size of 18 individuals (i.e., omitting B1 samples), ranged from 7.16 (in I2-98) to 8.51 (in I1-00) and was not correlated with population abundance ($P > 0.05$).

Across all populations, no linkage disequilibrium was detected between loci (all $P > 0.05$). Sixty percent of locus–population combinations showed significant deviations from HW expectations, after Bonferroni correction of the significance level. Four loci (*AeA12*, *AeB9*, *AeF10*, and *AeG5*) deviated from equilibrium conditions in almost all samples, whereas one locus (*AeH5*) met HW expectations in every single sample (Table 4). In all cases, the deviations were caused by heterozygote deficiencies. The most probable cause of the departures was null alleles identified using MICRO-CHECKER (van Oosterhout and others 2004). This was confirmed by the presence of nonamplifying individuals at these 4 loci despite several attempts. Null allele frequencies were estimated for each locus within each sample, and the allelic frequencies were adjusted to account for this bias (see Appendix). Null allele frequencies averaged across all sites were 0.20 ± 0.075 , 0.32 ± 0.113 , 0.27 ± 0.066 , and 0.38 ± 0.077 for *AeA12*, *AeB9*, *AeF10*, and *AeG5*, respectively (means \pm standard deviation). After the allele frequency adjustments, 7 exact tests conducted (20%) revealed deviations from HW proportions after Bonferroni correction. However, these deviations were not associated with a particular sample or loci.

This corrected data set could be used to test for recent population changes because the BOTTLENECK software allows for missing alleles in the genotypes. However, as a minimum of 10 individuals are needed to conduct this test, we excluded B1 samples (B1-98 and B1-00) from this analysis. Recent changes in effective population size ($P < 0.05$) were detected through heterozygote deficiencies in I1-00, I3-00, I2-00, and B2-97 under the SMM and TPM (Table 5). Using the uncorrected data set to conduct this test led to identical conclusions (data not shown).

Spatial and Temporal Genetic Structure

Over all pairwise comparisons, estimates of genetic differentiation were low with both genetic markers (Table 6). Within

Table 2. Sequence differences among the 37 haplotypes found in *Arhopala epimuta* samples. Dots indicate identical bases. The entire sequence of haplotype H1 was deposited in GenBank (accession number DQ491574)

	mtDNA CR 5' → 3'														12S RNA gene														
	4	6	6	8	9	9	3	5	6	6	7	7	8	8	2	2	2	2	2	2	3	3	3	3	3	3	4		
H1	A	—	—	T	A	A	A	T	A	A	T	A	A	T	T	A	G	G	A	A	A	—	—	C	G	A	A	G	T
H2	C
H3	.	T	A
H4	C	C
H5	.	T	A	A
H6	.	T	A	C	C
H7	C	A
H8	A	.	T	.	G	.	.	.
H9	.	T	A	C	A	.	.	.	A	G	.	.
H10	.	T	A	G
H11	G
H12	C	G
H13	A
H14	.	T	A	A	.	T
H15	C
H16	C
H17	G
H18	G
H19	C	.	.	.	G
H20	G
H21	C	A	A
H22	.	T	A	C
H23	C	A
H24	C	G
H25	.	T	A	C
H26	.	T	A	G
H27	.	.	C	C
H28	G
H29	A	A
H30	.	T	A	C	T	.	.	A
H31	C	A	G	.	.
H32	.	T	A	C	A
H33	G	.	.	C
H34	T
H35	.	.	.	G	A	.
H36	C	A	A	A
H37	G	T

regions at time *t* (Gunung Meratus in 2000, Sungai Wain in 1998 and in 2000), none of the pairwise comparisons were significant. Using the uncorrected microsatellite data set, 2 pairwise samples appeared genetically divergent: B2-97 and I3-00 ($F_{ST} = 0.012$, $P = 0.009$) and B2-97 and I2-00 ($F_{ST} = 0.008$, $P = 0.02$). Using the adjusted data set, these pairwise comparisons remained significant after Bonferroni correction of the significance level (B2-97 and I3-00, $F_{ST} = 0.004$, $P < 0.001$; B2-97 and I2-00, $F_{ST} = 0.002$, $P < 0.002$), whereas no other pairwise comparison was significant.

Using the mtDNA marker, 100% of the total variance was attributed to variation within the 7 samples (1-group AMOVA; global $\Phi_{ST} = 0.000$). Microsatellite single-locus AMOVAs conducted on adjusted allele frequencies

(including null alleles) gave similar findings. Within landscapes, no significant genetic divergence was found between sample years using either the mtDNA or the microsatellite markers.

Discussion

Microsatellite Markers in Lepidoptera and Null Alleles

Developing microsatellite markers in Lepidoptera is a major challenge (Meglécz and others 2004; Zhang 2004). Authors report small numbers of useful loci per species, ranging from 4 to 15 (Harper and others 2000; Caldas and others 2002; Cassel 2002; Flanagan and others 2002; Keyghobadi and others 2002; Williams and others 2002; Ji and others 2003;

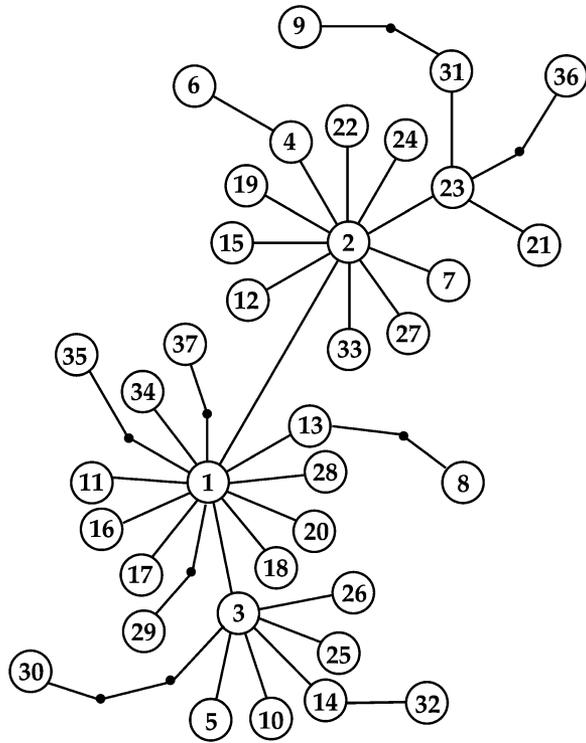


Figure 2. Minimum spanning network displaying evolutionary relationships among the 37 *Arbopala epimuta* mtDNA haplotypes (see Table 3 for haplotype distribution). Each circle corresponds to one haplotype, and each line between 2 haplotypes corresponds to one mutation (see Table 2 for reference). Small black circles indicate missing haplotypes.

Bezzerides and others 2004; but for recent exceptions, see Franck and others 2005; van't Hof and others 2005). The explanation partially lies in the presence of similar fragments found in flanking regions of different microsatellite loci, hence reducing the success of single-locus amplification (Megléc and others 2004; Zhang 2004; Fauvelot 2005). Indeed, locus *AeG5* (GenBank accession numbers AY974051) shared one of its flanking regions with at least 6 other loci (GenBank accession numbers DQ380839, DQ380841, DQ380843, DQ380845, DQ380849, and DQ380851).

As in the majority of studies using microsatellite markers in Lepidoptera (Megléc and Solignac 1998; Keyghobadi and others 1999, 2002, 2005; Harper and others 2000, 2003; Anthony and others 2001; Caldas and others 2002; Flanagan and others 2002; Ji and others 2003; Williams and others 2003; Bezzerides and others 2004; Franck and others 2005; van't Hof and others 2005), we found homozygote excesses when compared with HW expectations. Inbreeding and/or undetected population subdivision (Wahlund effect), both affecting all loci, selection, and/or the presence of null alleles (both locus specific), might create such deficiencies. In our study, 1 of the 5 loci met HW expectations,

and a notable set of individuals did not amplify at the 4 loci showing HW disequilibrium despite several attempts. These are indications of the presence of null alleles at the 4 loci in disequilibrium. Null alleles can be generated from poor primer annealing due to point mutations or indels in the flanking primer (Pemberton and others 1995), short allele dominance (i.e., differential amplification of size-variant alleles, Wattier and others 1998), or inconsistent DNA template quality. Because we did not sequence microsatellite-flanking regions, we cannot specify the mutation occurrence in the primer region. However, because 1) the null alleles were found over all classes of alleles, 2) null homozygotes were encountered in all loci/populations where deficiencies were observed, and 3) DNA extractions were reconducted for individuals with PCR failures without further success, it is very likely that null alleles identified in 4 of the 5 scored loci resulted from mishybridization of at least one primer.

Estimated null allele frequencies in our study (0.20–0.38) are high but in the range of null allele frequencies presented in Dakin and Avise (2004) based on 74 microsatellite loci from a wide range of organisms including Lepidoptera. They are furthermore similar to null allele frequencies obtained for microsatellite loci in other Lepidoptera, for example, the Adonis blue butterfly *Polyommatus bellargus* (from 0.03 to 0.2 at 5 loci; Harper and others 2003) and the Rocky Mountain Apollo butterfly *Parnassius smintheus* (from 0.03 to 0.34 at 7 loci; Keyghobadi and others 2005). Although null alleles lead to underestimated heterozygosity within samples, it is a minor source of error in estimating heterozygosity excess (Cornuet and Luikart 1996) and in parental assessments (Dakin and Avise 2004). Recently, O'Reilly and others (2004) and Peijnenburg and others (2006) showed that estimates of differentiation and the probability of detecting genetic differences among populations both diminished when locus heterozygosities were high and data corrected for null alleles. We found that adjusting our data set according to the presence of null alleles did not significantly modify our results concerning the detection of past population size changes in the samples. Likewise, we obtained similar population differentiation estimations using the mtDNA sequence data set and the uncorrected and the adjusted microsatellite data set.

Together, these findings suggest that null alleles likely do not have a major impact on our results, though we are aware that this survey should be conducted with more microsatellite loci in HW to conclude so.

Population Size Changes

The highly unpredictable nature of fires makes it impossible to design a perfect sampling scheme (comprising pre- and postdisturbance samples) to study the short-term impact of fires on the genetic diversity of butterfly populations. Among our 5 sampled landscapes, 2 were located in severely burned forests (B1 and B2). By chance, the Wanariset landscape (B2) was sampled just before the 1997/1998 ENSO event. In 1997, the Wanariset forest presented a mosaic of

Table 3. Distribution of mtDNA haplotypes and genetic diversity in *Arbopala epimuta* samples

	I1-00	I3-00	B1-98	B1-00	I2-98	I2-00	B2-97	Overall
H1	5	9	1	2	4	6	7	34
H2	5	4	1	1	2	3	3	19
H3	2	3			1	1		7
H4	2							2
H5	1							1
H6	1							1
H7	1							1
H8	1							1
H9	1							1
H10	1							1
H11	1					1		2
H12	1							1
H13		1			1	1		3
H14		1						1
H15		1						1
H16		1						1
H17		1						1
H18		1				1		2
H19			1					1
H20			1					1
H21				1				1
H22				2	2	1	1	6
H23					2			2
H24					1			1
H25					1			1
H26					1			1
H27						1		1
H28						1		1
H29						1		1
H30						1		1
H31						1		1
H32						1		1
H33							1	1
H34							1	1
H35							1	1
H36							2	2
H37							1	1
<i>N</i>	22	22	4	6	15	20	17	106
<i>H</i>	12	9	4	4	9	13	8	37
<i>b</i>	0.905	0.805	1	0.867	0.914	0.905	0.816	0.861
π	0.0047	0.0026	0.0036	0.0037	0.0038	0.0045	0.0042	0.0039
<i>k</i>	2.18	1.22	1.67	1.73	1.75	2.08	1.97	1.82
<i>D</i>	-1.38	-1.17	0.17	-0.06	-0.18	-1.56	-1.22	-1.98*
<i>b</i> -test	0.930	0.815	—	—	0.999	0.890	0.522	0.451

Number of individuals sequenced per landscape (*N*), total number of haplotypes (*H*), haplotype diversity (*b*, Nei 1987), nucleotide diversity (π , Nei 1987), mean number of pairwise nucleotide differences (*k*, Tajima 1983), Tajima's *D* statistic (*D*, Tajima 1989; **P* = 0.006), and *P* value associated with the *b*-test (Depaulis and Veuille 1998) conducted in DNASP.

forest in an advanced stage of regeneration together with primary dipterocarp forest (Slik and others 2002) because it had already been partially burned during the 1982/1983 ENSO event. Despite this past disturbance, *A. epimuta* was the second-most dominant species within Wanariset in 1997 (Cleary and Grill 2004), and its relative abundance was almost 3-fold that of the unburned and unlogged forest from Gunung Meratus sampled in 2000. The Wanariset forest was severely affected by the 1997/1998 ENSO fires (more than 90% of the forest burned, Cleary and Grill

2004), and no *A. epimuta* were recorded in this forest in 1998, 2000, and even 2004 (C Fauvelot and DFR Cleary, personal observations), further preventing the assessment of genetic diversity in this landscape after the fires.

After the 1997/1998 ENSO event, *A. epimuta* was recorded in proximate sampled landscapes within the Sungai Wain Protection Forest (ca. 20 km from Wanariset); abundance, however, within the burned landscape B1 was almost 5 times lower than in the adjoining unburned isolate I2. Because of the lack of sampling in Sungai Wain in 1997,

Table 4. Genetic diversity at 5 microsatellites loci in *Arhopala epimuta* samples

	I1-00	I3-00	B1-98	B1-00	I2-98	I2-00	B2-97
<i>N</i>	48	77	6	6	18	110	48
<i>AeA12</i>							
H_N	0.721	0.741	0.783	0.433	0.686	0.731	0.763
H_O	0.458	0.284	0.333	0.500	0.278	0.477	0.500
<i>f</i>	0.365*	0.617*	0.574	-0.154	0.595*	0.347*	0.345*
<i>AeB9</i>							
H_N	0.886	0.873	0.950	0.767	0.913	0.894	0.911
H_O	0.447	0.378	0.200	0.167	0.556	0.388	0.447
<i>f</i>	0.496*	0.566*	0.789	0.783	0.392*	0.567*	0.509*
<i>AeF10</i>							
H_N	0.736	0.689	0.833	0.733	0.746	0.698	0.798
H_O	0.489	0.263	0.167	0.667	0.353	0.426	0.283
<i>f</i>	0.335*	0.618*	0.800	0.091	0.527*	0.390*	0.646*
<i>AeG5n</i>							
H_N	0.693	0.530	0.625	0.833	0.761	0.664	0.708
H_O	0.239	0.192	0.400	0.000	0.294	0.182	0.316
<i>f</i>	0.655*	0.638*	0.360	1.000	0.614*	0.726*	0.554*
<i>AeH5</i>							
H_N	0.675	0.677	0.700	0.683	0.706	0.687	0.629
H_O	0.565	0.693	0.833	0.667	0.722	0.578	0.596
<i>f</i>	0.162	-0.024	-0.190	0.024	-0.023	0.158	0.053
Overall							
H_N	0.742	0.702	0.778	0.690	0.762	0.735	0.762
H_O	0.440	0.362	0.387	0.400	0.441	0.410	0.428
<i>f</i>	0.407*	0.484*	0.503*	0.420*	0.422*	0.442*	0.438*
All/loc	12.2	12.4	4	3.8	7.2	13.6	10.2
Ac(18)	8.51	7.51	—	—	7.16	8.24	7.96

N = number of individuals scored per sample, H_N = unbiased gene diversity (Nei 1987), H_O = observed heterozygosity, *f* = inbreeding coefficient of Weir and Cockerham (1984), All/loc = mean number of alleles per locus, and Ac(18) = allelic richness based on 18 individuals (El Mousadik and Petit 1996).

* Non conformance to HW proportions.

we cannot be sure that the lower abundance observed in the burned landscape is a consequence of the fires. However, the landscapes B1 and I2 were both sampled in an originally large unburned forest prior to the disturbance (the Sungai Wain

Table 5. Results of the Wilcoxon signed-rank tests computed using BOTTLENECK (Piry and others 1999) for heterozygosity excess or deficiency under TPM and SMM for 5 *Arhopala epimuta* samples. Significant *P* values are represented in bold

	No. loci		<i>P</i> value	
	TPM	SMM	TPM	SMM
I1-00				
Deficiency	4	5	0.031	0.016
Excess	1	0	—	—
I3-00				
Deficiency	5	5	0.016	0.016
Excess	0	0	—	—
I2-98				
Deficiency	2	2	0.594	0.406
Excess	3	3	0.500	0.688
I2-00				
Deficiency	5	5	0.016	0.016
Excess	0	0	—	—
B2-97				
Deficiency	4	5	0.031	0.016
Excess	1	1	—	—

Protection Forest), so that it is very likely that similar abundances of *A. epimuta* were observed in both landscapes prior to the 1997/1998 ENSO disturbance. In fact, abundance estimates within the unburned landscape I2 in 1998 and in 2000 were more or less similar among all sampled transects (data available from the authors on request). Therefore, because 1) *A. epimuta* abundance was lower in B1 than I2, 2) a population expansion was observed postdisturbance in B1 (the relative abundance almost tripled between 1998 and 2000), and 3) the fires exterminated *A. epimuta* populations in the proximate Wanariset forest that was also severely burned, we can deduce that the fires caused *A. epimuta* population bottlenecks within the burned forest of Sungai Wain.

According to theoretical predictions with respect to the genetic consequences of population bottlenecks, reduced genetic diversity should be observed in *A. epimuta* populations affected by the 1997/1998 ENSO event. However, we were not able to obtain reliable estimates of genetic diversity within the burned landscape sampled after the fires because of the low numbers of recorded *A. epimuta* within this landscape (4 in B1-98 and six in B1-00).

A relative abundance increase was observed in the small unburned isolate within Sungai Wain (I2) between 1998 and 2000 (increase by a factor of 4 in a 2-year period). This increase in *A. epimuta* abundance could result from 1) random

Table 6. Pairwise F_{ST} estimates using 5 microsatellite loci (above the diagonal) and Φ_{ST} estimates using mtDNA CR sequences (below the diagonal) between *Arbopala epimuta* samples

	Gunung Meratus		Sungai Wain				Wanariset
	I1-00	I3-00	B1-98	B1-00	I2-98	I2-00	B2-97
I1-00	—	0.000	0.000	0.014	0.000	0.000	0.007
I3-00	0.027	—	0.000	0.029	0.006	0.000	0.012*
B1-98	0.000	0.046	—	0.000	0.000	0.000	0.000
B1-00	0.000	0.082	0.000	—	0.015	0.027	0.008
I2-98	0.000	0.015	0.009	0.000	—	0.000	0.000
I2-00	0.000	0.000	0.000	0.000	0.000	—	0.008*
B2-97	0.019	0.025	0.000	0.009	0.023	0.001	—

* $P < 0.05$.

fluctuations associated either with variation in the reproductive success of this species or with the sampling method, 2) a recovery after a population bottleneck, and/or 3) an influx of individuals from the surrounding burned forests. However, because a population size change was reflected in the microsatellite data set in I2-00, and considering that abundance was estimated using a standardized method (i.e., no sampling bias; Cleary 2003), this expansion probably did not result from random fluctuations. Moreover, a significant departure from neutrality was observed in this landscape in 1998 using the mtDNA sequence data set, suggesting that *A. epimuta* present in the pristine isolate of Sungai Wain forest were affected by the drought (the unburned isolate was surrounded by burned forest and suffered severe drought; Slik and Eichhorn 2003). Also, the occurrence of newly recorded mitochondrial haplotypes in I2 in 2000 suggests immigration. Nevertheless, because we lack a pre-1997/1998 ENSO sampling, we cannot be sure that the population expansion corresponds to a recovery because we cannot totally exclude the possibility of a sampling bias in explaining observed variations in haplotype distributions between 1998 and 2000 (not all *A. epimuta* were sequenced).

Within the Sungai Wain Protection Forest, a much slower population expansion was observed in the burned landscape B1 than the unburned I2. The explanation is certainly that fires affected most of the larval host plants of *A. epimuta* within B1 when compared with the proximate I2 (Cleary and Priadhati 2005). This also happened in Wanariset, leading to the local extirpation of *A. epimuta* there. However, the lack of a pre-ENSO sample within Sungai Wain (because of the unpredictability of the forest fires) weakens our conclusions. Nevertheless, the contrasted situation observed in nearby habitats differentially affected (by fires or by drought) is an indication of the importance of unburned forest for the survival of *A. epimuta* populations.

Within the unburned landscapes I1 and I3 (both sampled in 2000 in Gunung Meratus region) and the burned landscape B2 (sampled in 1997 in Wanariset forest), genetic evidence of population expansions was detected using the microsatellite data set. The population densities of *A. epimuta* prior to sampling within these landscapes are unknown. However, I3 and

B2 are landscapes that suffered from some form of disturbance; I3 was commercially logged in 1993–1994, and B2 was a primary forest that partially burned during the less severe 1982/1983 ENSO event (Slik and others 2002). Therefore, population expansions may have followed these intermediate disturbances in both landscapes. Nevertheless, the population sampled in the pristine Gunung Meratus forest (I1) was expected to be at mutation-drift equilibrium because no major disturbance was recorded prior to 2000 (Cleary and others 2004). As null alleles may cause an underestimation of the heterozygosity within sample, the observation of heterozygosity deficiencies should be interpreted with caution. In addition, Harper and others (2003) found that 12.5% of populations documented as having remained stable displayed excesses of heterozygosity, indicating recent bottlenecks. Yet, because we lack population size estimations of the *A. epimuta* population in I1 prior to 2000, we cannot conclude that a recent population expansion resulted from migrants from proximate burned landscapes, as in I2, or if the results of the Wilcoxon signed-rank test are biased by low loci number coupled with the occurrence of null alleles.

Nevertheless, population size changes (both reduction and expansion) were detected within *A. epimuta* populations sampled from the Balikpapan–Samarinda region of East Kalimantan, and the demographic bottlenecks were likely the consequences of the 1997/1998 ENSO disturbance.

Altogether, our results show that even though *A. epimuta* local populations may be extirpated by the disturbance, the proximate remnant populations located in patchy habitat may “gain” from the subsequent fragmentation by receiving immigrants from disturbed sites. Under this model, known as the refugee model (Porter 1999), genetic reorganization by movements of refugees (immigrants) causes deviations from genetic equilibrium, increasing genetic variation within the remaining populations and decreasing differentiation among them. Further investigations at larger spatial scales are therefore needed to infer the consequences of the 1997/1998 ENSO disturbance as we now need to disentangle the levels of genetic diversity resulting from the refugee model from the original levels of genetic diversity within undisturbed forests.

Appendix A

Table A1. Within-sample microsatellite allele frequencies observed at 5 microsatellite loci and microsatellite allele frequencies adjusted for null allele occurrence according to Brookflied (1996)

Locus allele	I1-00 (48)		I3-00 (77)		B1-98 (6)		B1-00 (6)		I2-98 (18)		I2-00 (110)		B2-97 (48)	
	Obs.	Adj.	Obs.	Adj.	Obs.	Adj.	Obs.	Adj.	Obs.	Adj.	Obs.	Adj.	Obs.	Adj.
<i>AeA12</i>														
144			0.014	0.009										
146	0.135	0.115	0.169	0.115	0.333	—			0.111	0.086	0.225	0.188	0.156	0.134
150											0.009	0.008		
151			0.007	0.005										
152	0.208	0.178	0.237	0.160	0.167	—			0.389	0.300	0.211	0.176	0.156	0.134
153	0.010	0.009					0.083	—						
154	0.188	0.160	0.169	0.115	0.083	—	0.167	—	0.083	0.064	0.151	0.126	0.219	0.187
156	0.438	0.373	0.392	0.266	0.417	—	0.750	—	0.417	0.322	0.395	0.329	0.385	0.330
158	0.021	0.018									0.005	0.004	0.031	0.027
160													0.021	0.018
162													0.031	0.027
164			0.007	0.005										
174											0.005	0.004		
176			0.007	0.005										
Null		0.148		0.322						0.228		0.166		0.144
<i>AeB9</i>														
118											0.005	0.003		
124											0.005	0.003	0.011	0.008
126													0.011	0.008
128	0.043	0.032	0.007	0.005					0.028	0.023				
130	0.011	0.008											0.011	0.008
132			0.020	0.014	0.100	0.048			0.028	0.023				
134	0.021	0.016									0.015	0.009		
136			0.014	0.009							0.005	0.003		
138	0.032	0.024									0.056	0.033	0.011	0.008
140	0.021	0.016							0.056	0.046	0.026	0.015		
142	0.021	0.016	0.014	0.009							0.015	0.009	0.021	0.016
144	0.011	0.008	0.014	0.009							0.015	0.009		
146	0.021	0.016	0.014	0.009					0.083	0.069	0.077	0.045	0.032	0.023
148	0.106	0.079	0.101	0.069	0.200	0.096	0.500	0.353	0.222	0.184	0.056	0.033	0.202	0.148
150	0.064	0.047	0.142	0.097	0.300	0.144			0.056	0.046	0.117	0.070	0.128	0.093
152	0.298	0.220	0.277	0.189	0.200	0.096	0.083	0.059	0.194	0.161	0.260	0.154	0.085	0.062
154	0.064	0.047	0.135	0.092	0.200	0.096	0.167	0.118	0.056	0.046	0.087	0.051	0.085	0.062
156	0.053	0.039	0.061	0.042					0.083	0.069	0.051	0.030	0.064	0.047
158	0.096	0.071	0.041	0.028			0.250	0.177			0.066	0.039	0.128	0.093
160	0.021	0.016	0.027	0.018					0.056	0.046	0.026	0.015	0.075	0.054
162	0.011	0.008	0.020	0.014					0.056	0.046	0.026	0.015	0.011	0.008
164			0.007	0.005							0.026	0.015	0.021	0.016
166	0.011	0.008												
168	0.011	0.008	0.007	0.005									0.043	0.031
170											0.005	0.003		
172	0.011	0.008												
174									0.028	0.023	0.010	0.006		
176	0.021	0.016	0.014	0.009					0.056	0.046	0.026	0.015		
178			0.047	0.032							0.010	0.006	0.043	0.031
180	0.043	0.032	0.027	0.018							0.015	0.009	0.011	0.008
182	0.011	0.008	0.014	0.009										
186													0.011	0.008
Null		0.261		0.318		0.520		0.294		0.172		0.407		0.270
<i>AeF10</i>														
121	0.011	0.009	0.007	0.005					0.059	0.041	0.023	0.019	0.054	0.036
123	0.021	0.017	0.007	0.005										
124	0.479	0.391	0.520	0.378	0.417	0.285	0.500	—	0.412	0.287	0.509	0.409	0.380	0.250
127	0.096	0.078	0.099	0.072			0.250	—	0.206	0.143	0.097	0.078	0.109	0.071
128											0.005	0.004		

Table A1. Continued

Locus allele	I1-00 (48)		I3-00 (77)		B1-98 (6)		B1-00 (6)		I2-98 (18)		I2-00 (110)		B2-97 (48)	
	Obs.	Adj.	Obs.	Adj.	Obs.	Adj.	Obs.	Adj.	Obs.	Adj.	Obs.	Adj.	Obs.	Adj.
130	0.043	0.035	0.105	0.077							0.051	0.041	0.033	0.021
133	0.149	0.122	0.151	0.110	0.250	0.171	0.083	—	0.265	0.184	0.167	0.134	0.207	0.136
136	0.032	0.026	0.033	0.024	0.167	0.114	0.083	—	0.029	0.021	0.060	0.048	0.054	0.036
139	0.085	0.070	0.053	0.038	0.167	0.114	0.083	—	0.029	0.021	0.046	0.037	0.087	0.057
142	0.032	0.026									0.028	0.022	0.033	0.021
145	0.011	0.009	0.007	0.005									0.044	0.029
148	0.043	0.035	0.013	0.010							0.014	0.011		
163			0.007	0.005										
Null		0.183		0.273		0.317				0.304		0.198		0.343
<i>AeG5</i>														
73			0.021	0.014										
76	0.022	0.015							0.059	0.039	0.025	0.015	0.040	0.020
77	0.011	0.007									0.010	0.006		
78	0.022	0.015	0.007	0.005							0.010	0.006		
79	0.011	0.007	0.034	0.023							0.015	0.009	0.145	0.073
80	0.011	0.007												
82	0.033	0.022	0.034	0.023					0.177	0.117	0.091	0.052	0.158	0.080
83			0.007	0.005							0.005	0.003		
84	0.022	0.015									0.010	0.006		
85	0.033	0.022	0.089	0.061	0.100	—	0.250	—	0.029	0.020	0.061	0.035	0.079	0.040
87									0.059	0.039	0.030	0.017		
88	0.533	0.357	0.678	0.464	0.600	—	0.500	—	0.471	0.312	0.566	0.324	0.500	0.253
91	0.152	0.102	0.062	0.042	0.300	—			0.088	0.059	0.066	0.038	0.040	0.020
94	0.087	0.058	0.014	0.009					0.059	0.039	0.040	0.023	0.026	0.013
97	0.033	0.022	0.021	0.014							0.005	0.003		
100	0.022	0.015	0.021	0.014					0.059	0.039	0.010	0.006		
103	0.011	0.007	0.014	0.009			0.250	—			0.035	0.020	0.013	0.007
106											0.010	0.006		
109											0.005	0.003		
112											0.005	0.003		
Null		0.330		0.316						0.337		0.427		0.495
<i>AeH5</i>														
155	0.011	—												
167			0.007											
170	0.011	—	0.020	—					0.083	—	0.028	—		
171			0.007	—									0.011	—
172											0.009	—		
173	0.337	—	0.313	—	0.500	—	0.500	—	0.306	—	0.326	—	0.298	—
174	0.011	—	0.013	—							0.028	—	0.043	—
175			0.007	—										
176	0.457	—	0.467	—	0.250	—	0.333	—	0.444	—	0.445	—	0.532	—
177	0.087	—	0.087	—	0.167	—	0.083	—	0.056	—	0.023	—	0.021	—
178											0.009	—		
179	0.054	—	0.053	—	0.083	—	0.083	—	0.111	—	0.101	—	0.053	—
182											0.014	—		
185	0.033	—	0.020	—							0.018	—	0.032	—
188			0.007	—										
206													0.011	—

Obs., observed; adj., adjusted.

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