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Cécile Fauvelot, D F R Cleary, S B J Menken. Short-term impact of disturbance on genetic diversity and structure of Indonesian populations of the butterfly *Drupadia thedai* in East Kalimantan. *Molecular Ecology*, 2006, 15, pp.2069 - 2081. 10.1111/j.1365-294x.2006.02920.x . ird-03044126

HAL Id: ird-03044126

<https://ird.hal.science/ird-03044126>

Submitted on 7 Dec 2020

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Short-term impact of disturbance on genetic diversity and structure of Indonesian populations of the butterfly *Drupadia theda* in East Kalimantan

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Abstract

We investigated the short-term impact of disturbance on genetic diversity and structure of the tropical butterfly *Drupadia theda* Felder (Lepidoptera: Lycaenidae). Populations were sampled from five landscapes in East Kalimantan (Borneo, Indonesia) which were differentially disturbed by selective logging and the 1997/1998 El Niño Southern Oscillation (ENSO)-induced drought and fires. Sampling occurred before (in 1997) and after the forest fires (in 1998, 1999, 2000, and 2004). *Drupadia theda* populations underwent serious population size reductions following the 1997/1998 ENSO event. For a total of 208 individuals, we sequenced a 509-bp segment of mtDNA containing the control region *plus* the 5' end of the 12S rDNA gene. Haplotype diversity in *D. theda* populations ranged from 0.468 to 0.953. Just after the 1997/1998 ENSO event, number of recorded individuals and genetic diversity were very low in *D. theda* populations sampled in the two severely burned areas and in a small pristine forest fragment that was surrounded by burned forest and thereby affected by drought. Interestingly, higher levels of genetic diversity were observed in logged forest compared to proximate pristine forest. After 1998, the genetic composition within the three ENSO-disturbed areas diverged. In the twice-burned forest, the genetic diversity in 1999 already approached pre-fire levels, while it remained nearly unchanged in proximate once-burned forest. Our data suggest that the 1997/1998 ENSO-induced drought and fires caused massive reductions in the genetic diversity of *D. theda* and that population recoveries were linked to their geographical position relative to patches of unburned forest (and thus to source populations).

Keywords: bottleneck, disturbance, gene flow, genetic diversity, Lepidoptera, mtDNA

Received 10 November 2005; revision accepted 22 January 2006

Introduction

Disturbance is often cited as the principal factor regulating species diversity. It has been described as a force, often abrupt and unpredictable, that kills or badly damages organisms and alters the availability of resources (Mackey & Currie 2000). Different forms of disturbance can have different impacts on biotic assemblages with respect to

species richness. In tropical rainforests, the two prevalent forms of disturbance are logging and El Niño Southern Oscillation (ENSO)-induced disturbances. Previous studies have found that, despite often causing severe structural damage, legal logging seldom leads to reductions in species diversity (Cannon *et al.* 1998; Willott *et al.* 2000; Lewis 2001; Cleary 2003) and may be considered as an intermediate disturbance (i.e. intermediate in intensity or in frequency). On the contrary, drought and fires induced by ENSO events are a major disturbance because they can dramatically modify natural habitats over very large spatial and temporal scales (Holmgren *et al.* 2001; Siebert *et al.* 2001; Laurance 2003). Effects of disturbance on tropical species diversity have been investigated in quite a number of organisms, such as flowering plants (Van Nieuwstadt

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et al. 2001; Brown & Gurevitch 2004; Cleary & Priadhati 2005), birds (Thiollay 1992), mammals (Malcolm & Ray 2000), and insects (Willott *et al.* 2000; Lewis 2001; Cleary 2003; Cleary & Genner 2004; Cleary *et al.* 2004). However, little is known about its effect on genetic diversity within species.

The Indonesian province of East Kalimantan (Borneo) was severely affected by the 1997/1998 ENSO event (more than 5 million hectares burned), which was by far the most severe and widespread recorded event in Borneo (Harrison 2000; Siegert *et al.* 2001). Butterflies in Borneo have previously been extensively studied in terms of community responses to both logging and ENSO-induced droughts and fires (Willott *et al.* 2000; Cleary 2003; Hamer *et al.* 2003; Cleary & Genner 2004; Cleary & Mooers 2004; Cleary *et al.* 2004). In East Kalimantan, the fires created a mosaic of unburned forests (some of which were affected by drought), isolating local populations of butterfly species and reducing species richness in the affected areas (Cleary 2003; Cleary & Genner 2004). Higher levels of species richness were observed in logged forests compared to pristine forest (Cleary 2003, 2004). However, we do not know the consequences of such disturbance at the intraspecific level.

Genetic diversity using the mitochondrial DNA control region (CR; also called D-loop) was assessed in *Drupadia theda* Felder (Lepidoptera: Lyceanidae), a rather common rainforest lycaenid that feeds on a wide range of plant families including Fabaceae, Guttiferae, Meliaceae, Rubiaceae, Meliaceae, Clusiaceae, and Sapindaceae (Elliot 1992; Igarashi & Fukada 1997, 2000; Robinson *et al.* 2001; K. Fiedler, personal communication). This species can thus be considered a generalist with respect to host plant use. However, *D. theda* is considered a specialist in terms of myrmecophily as it is obligatorily and specifically associated with *Crematogaster* ant species (Seufert & Fiedler 1996), which often form very large and quite territorial colonies. Egg laying typically occurs in close physical contact with host ants, mostly in small groups, and larvae are attended from the first instar onwards (K. Fiedler, personal communication). The average development time from egg to adult of *D. theda* is 28.3 days (Seufert & Fiedler 1996). *Drupadia theda* populations underwent serious population size reductions in East Kalimantan following the 1997/1998 ENSO-induced fires (Cleary 2003; Cleary & Grill 2004). These population size changes are expected to leave a genetic footprint, different from the one expected for constant size populations, which can be revealed by DNA sequencing (Slatkin & Hudson 1991; Rogers & Harpending 1992). Theoretically, population size reductions are predicted to result in a loss of genetic diversity (Wright 1931; Frankham 1995) and an increase of genetic differentiation between populations, due to the increased influence of genetic drift (Chakraborty & Nei 1976). In addition, small and isolated populations are vulnerable to inbreeding depression (Sakai *et al.* 2001; Frankham *et al.* 2002), that might reduce

their evolutionary potential and increase their risk of extinction (Frankham 1995; England *et al.* 2003). Historically documented bottlenecks, such as those resulting from the 1997/1998 ENSO event, provide unique opportunities to study the consequences of such events on genetic variability and the evolutionary potential of natural populations.

We investigated the short-term impact of disturbance on genetic diversity and composition of differentially affected *D. theda* populations. The analysed populations originated from pristine, logged, and burned forests in different landscapes in East Kalimantan, which were sampled before (in 1997) and after the ENSO event (in 1998, 1999, 2000, and 2004). To our knowledge, this is the first study investigating the short-term impact of logging and ENSO-induced disturbance on the genetic diversity of a tropical rainforest species, with pre- and postdisturbance samples analysed.

Materials and methods

Sampling

Specimens of *Drupadia theda* were collected during surveys conducted in 1997 (before the fires), 1998, 1999, 2000, and 2004 in the Balikpapan-Samarinda region of East Kalimantan, Indonesian Borneo (Fig. 1). Five differentially disturbed landscapes were sampled. The three landscapes in unburned isolates (I1, I2, I3) and the remaining two in burned forests (B1, B2) were located in the 5.2 million ha of East Kalimantan that changed from a habitat mosaic of primary forest with areas of secondary forest to an area dominated by secondary (burned) forest with only remnant unburned patches (Siegert *et al.* 2001). All landscapes are described in detail, in terms of location and dominant vegetation, in Cleary (2003). Briefly, I1 (unlogged primary) and I3 (logged in 1993/1994) landscapes are located in a 108 000 ha large unburned isolate located in part of the ITCI (International Timber Concessions Indonesia) and including the Gunung Meratus Protected Forest Reserve (c. 30 000 ha). The I2 landscape is located in a small, 3500-ha unburned primary isolate that is all that remains of the Sungai Wain Protected Forest; 6500 ha of the original 10 000 ha burned during the 1997/1998 ENSO event. The once-burned landscape B1 is located in the burned part of the Sungai Wain Nature Reserve and burned for the first time during the 1997/1998 ENSO event. The twice-burned landscape B2 was partially burned during the 1982/1983 ENSO event and severely burned during the 1997/1998 ENSO event; it is located in the Wanariset Samboja Research Forest. None of the burned landscapes have been commercially logged although they may have been subjected to unrecorded illegal logging.

Specimens of *D. theda* were sampled along 300-m transects as described in Cleary (2003). Individuals were immediately killed, after which three legs were removed and stored in 96% ethanol. Each individual was assigned a unique code

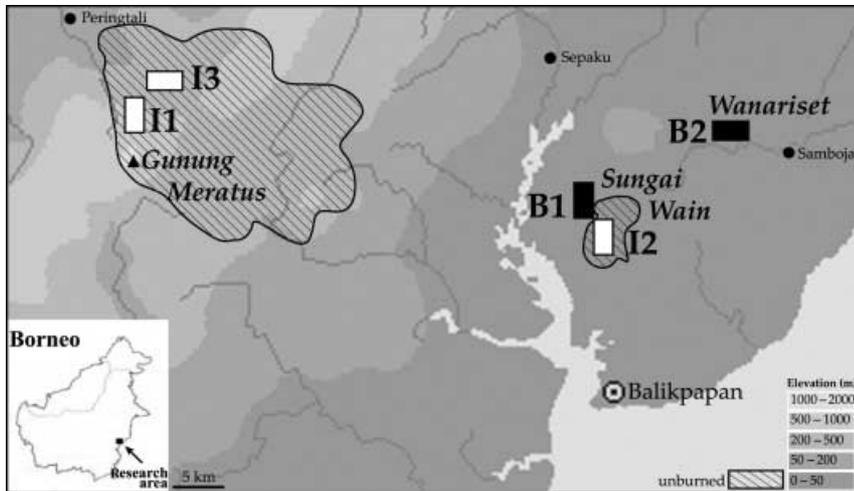


Fig. 1 Map of the research area, located in the Balikpapan-Samarinda region of East Kalimantan (Indonesian Borneo) showing location of landscapes in which individuals of *Drupadia theda* were sampled. Black rectangles represent burned landscapes; white rectangles represent unburned isolates. I1 and I3 landscapes are located in the Gunung Meratus region and were sampled in 2000. B1 and I2 landscapes are located in the Sungai Wain region and were sampled in 1998 and in 2000. B2 is located in the Wanariset region and was sampled in 1997, 1998, 1999, 2000, and 2004.

that was recorded on the tube containing the legs and the papillote with the butterfly. Voucher specimens were preserved (using silica gel) and have been deposited in the Zoological Museum of the University of Amsterdam (ZMA), the Netherlands.

Molecular analysis

Genomic DNA was isolated from one leg of each individual using a cetyltrimethyl ammonium bromide (CTAB) protocol (Hillis & Moritz 1990). Amplifications of the mtDNA control region (CR) plus a fragment of the 5' end of the 12S rDNA gene were conducted using primers SeqLepMet and LepAT2B (Vila & Björklund 2004). Polymerase chain reaction (PCR) was performed in 25 μ L containing 1 \times PCR buffer (HT Biotechnology), 3 mM MgCl₂, 0.12 mM of each dNTP, 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): 2-min denaturation at 94 $^{\circ}$ C; 35 cycles of 1 min at 94 $^{\circ}$ C, 1 min at 54 $^{\circ}$ C, and 1 min at 65 $^{\circ}$ C; and a final extension of 5 min at 65 $^{\circ}$ C. Amplified fragments were purified with the Boom procedure (Boom *et al.* 1990) and cycle sequenced in both directions with either SeqLepMet or LepAT2B using BigDye Terminator sequencing kit (Applied Biosystems). Sequences were run on an ABI 3100 Genetic Analyser (Applied Biosystems). Forward and reverse sequences were compared for each individual in BIOEDIT (Hall 1999) and a single sequence was defined for each individual. Individual sequences were then aligned by eye in GENEDOC (Nicholas & Nicholas 1997).

Data analysis

Haplotypes were identified and their relative frequency within populations calculated using ARLEQUIN version

2.000 (Schneider *et al.* 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 Inc.). Pairwise distances between haplotypes or groups of haplotypes were estimated according to the uncorrected p distance using MEGA version 2.1 (Kumar *et al.* 1993). 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).

Genetic diversity within populations was measured as haplotype diversity (H_d , Nei 1987), nucleotide diversity (π , Nei 1987), and mean number of pairwise nucleotide differences between individuals (k , Tajima 1983) using DNASP 4.0 (Rozas *et al.* 2003). Tajima's D test (Tajima 1989) was conducted within each sample separately as well as within regions per year (by pooling samples), viz., Gunung Meratus (I1 + I3) in 2000, and Sungai Wain (B1 + I2) in 1998, and in 2000. Observed haplotype diversities were compared to the empirical distribution of this index generated using coalescent simulations in order to conduct the haplotype diversity test (H test; Depaulis & Veuille 1998), performed in DNASP. The 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 H_d , obtained from 10 000 simulations under the neutral coalescent process and given the number of segregating sites observed in each sampled population (the model therefore considers that the number of segregating sites is fixed and that mutations are uniformly distributed, at random, along lineages). DNASP thus estimates the probability of obtaining lower or higher values of H_d , computed from the simulation ($H_{d_{sim}}$), than the one observed in each sample ($H_{d_{obs}}$). These simulations were conducted within each sampled population and within the regions, pooling samples as described above.

Analyses of the spatial and temporal genetic population structure were performed with 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 (Excoffier *et al.* 1992) and the level of significance was adjusted according to the sequential Bonferroni correction for $\alpha = 0.05$ (Rice 1989). The matrix of pairwise Φ_{ST} was visualized by nonmetric multidimensional scaling (MDS) using the program XLSTAT® (Addinsoft S.A.R.L.). The fit of the data in two dimensions was measured with a stress factor (Kruskal & Wish 1978). The spatial distribution of genetic variation was examined using an analysis of molecular variance (AMOVA; Excoffier *et al.* 1992). AMOVAs were performed to test the null hypothesis that genetic variation was not associated (i) with temporal variation within sampled landscapes and (ii) with spatial structure in 2000 according to three regions: Gunung Meratus (I1 + I3), Sungai Wain (B1 + I2), and Wanariset (B2).

Results

Sequence analysis

A total of 208 individuals of *Drupadia theda* sampled from 11 spatial and temporal samples were analysed (Table 1). The percentage of individuals analysed compared to the number of recorded individuals during surveys varied from 4% to 63%. The amplified mtDNA fragment, varying from 502 to 509 bp, contained the control region (CR; 390–397 bp) plus a fragment of 112 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 A (the poly thymidine stretch at

the 5' end of the control region), within region D (the [TA]_n-like stretch), and within the homopolymer described in Vila & Björklund (2004) (Table 2). Because length polymorphism observed in region D originated from dinucleotide indels ([TA] indels, bp 96–101, Table 2), each of these dinucleotide indels were treated as a single base substitution. Among the 208 individuals sequenced, we identified 42 different haplotypes, distinguished by 39 polymorphic sites, from which eight corresponded to indels and 15 were singletons (Table 2). Of the 39 polymorphic sites, five were found in the 12S gene and only one site in the CR presented multiple substitutions (bp 283). Among haplotypes, the overall mean uncorrected sequence divergence was 1.31% and haplotype pairwise divergence ranged from 0.2 to 3.16%. Considered over all individuals, adenine and thymine contents were on average 49.1 and 43.6%, respectively. Haplotype diversity was 0.882, nucleotide diversity was 0.0094, and the mean number of pairwise differences between two individuals for the entire data set was 4.75.

The minimum spanning network revealed a subdivision of the 42 haplotypes into three groups (Fig. 2). Groups 1 and 2 were $1.5 \pm 0.4\%$ divergent, and groups 1 and 3 were $2.5 \pm 0.6\%$ (average uncorrected p distance). The haplotypes from group 3 (H6, H8, H9, and H11) were exclusively found in seven individuals from the Gunung Meratus region (six individuals from I3-00 and one from I1-00; Table 3). Haplotypes H8, H9, and H11 were all length variants of H6. In order to verify if these seven individuals could belong to a cryptic species (these individuals were not morphologically distinct from the others based on their wing patterns), we sequenced a 757-bp fragment of the mtDNA cytochrome oxidase subunit 1 region (COI) for two individuals carrying haplotype H6, for one individual carrying haplotype H1 (the most common haplotype), and for one individual of *Drupadia ravindra* (closely related to

Table 1 Sampling information. The total number of recorded *Drupadia theda* as well as the number of sequenced *D. theda* is given within each population. Ind./transect is the adjusted number of individuals recorded per transect and per day. Population names are given according to the sampled landscape (see Fig. 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 sampled in an originally pristine forest that burned during 1997/1998 ENSO

Population name	Sampled region	Sampled year	Forest status	Recorded individuals	No. of sequenced individuals	Ind./transect
I1-00	Gunung Meratus	2000	pristine	581	24	5.28
I3-00	Gunung Meratus	2000	logged	214	22	2.28
B1-98	Sungai Wain	1998	burned once	20	11	0.39
B1-00	Sungai Wain	2000	burned once	96	24	1.09
I2-98	Sungai Wain	1998	pristine	34	19	0.76
I2-00	Sungai Wain	2000	pristine	175	20	1.70
B2-97	Wanariset	1997	burned once	48	24	0.98
B2-98	Wanariset	1998	burned twice	4	2	0.08
B2-99	Wanariset	1999	burned twice	40	15	0.82
B2-00	Wanariset	2000	burned twice	249	25	2.27
B2-04	Wanariset	2004	burned twice	35	22	0.52

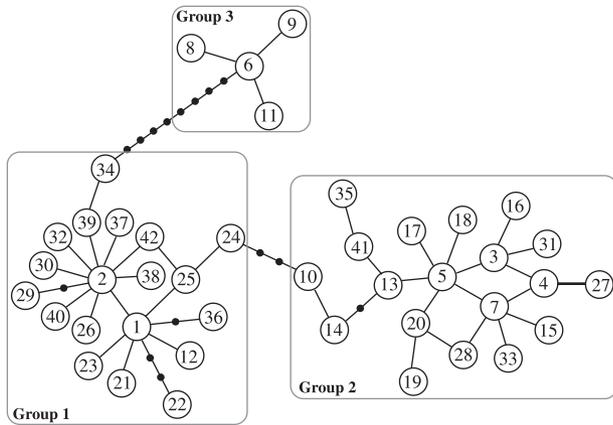


Fig. 2 Minimum-spanning network displaying evolutionary relationships among the 42 *Drupadia theda* mtDNA haplotypes (see Table 3 for haplotype distribution). Each circle corresponds to one haplotype; each line between two haplotypes corresponds to one mutation (see Table 2 for reference). A small black circle indicates a missing, one-mutational step haplotype.

Haplotypes H1 and H2 (length variants) were carried by 44% of the individuals from all sampled landscapes. Seven haplotypes were shared in at least two regions (Gunung Meratus, Sungai Wain or Wanariset), representing 66% of the individuals, while the rest of the individuals carried haplotypes that were restricted to a particular region (i.e. 35 haplotypes; see Table 3). Within landscapes, the number of haplotypes ranged from one (in B2-98) to 15 (in B2-97). Sample B2-98 was excluded from further analysis because only two individuals were collected in the Wanariset forest in 1998. However, it should be noted that this low sample size was the consequence of the low abundance of butterflies observed in this landscape in 1998 (0.08 individuals vs. nearly one individual per transect per day in 1997, with similar sampling intensity; see Table 1).

Genetic diversity and neutrality tests

Within landscapes, haplotype diversity ranged from 0.468 (for I2-00) to 0.953 in B2-97 (Table 4). The highest values of gene diversity were found in B2-97 and I3-00, followed by I1-00 and B2 sampled after 1997, and the lowest values were found in Sungai Wain, both in 1998 and 2000 (Fig. 3). Haplotype diversity was not correlated with the mean number of individuals per transect per day (Spearman's coefficient = 0.394, $P = 0.260$; Fig. 4) nor the numbers of recorded individuals within landscapes (Spearman's coefficient = 0.382, $P = 0.276$), nor the sample size (Spearman's coefficient = 0.526, $P = 0.123$). Samples B1-98 and I2-00 were composed of individuals carrying haplotype H1 (the most common one) and length variants associated with H1 (i.e. H2, H21, and H26).

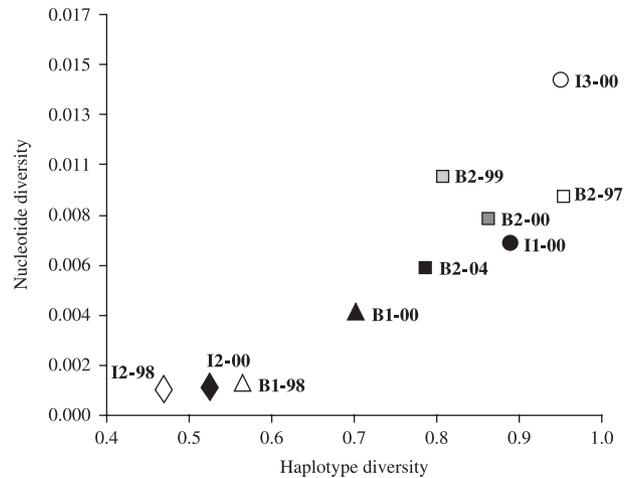


Fig. 3 Genetic diversity (presented as haplotype diversity, H_d , vs. nucleotide diversity, π) within *Drupadia theda* sampled populations.

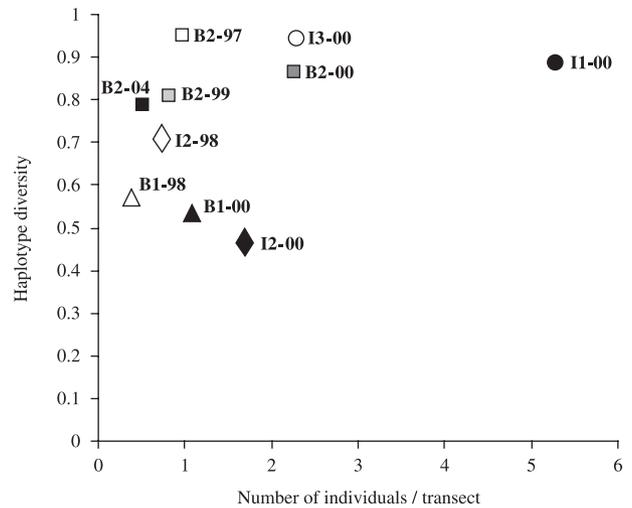


Fig. 4 Relation between the observed haplotype diversity and the number of individuals of *Drupadia theda* recorded per transect and per day within each sample (Spearman's coefficient = 0.394, $P = 0.260$).

Within the population from the burned Wanariset forest (B2), the genetic diversity remained more or less stable through time (with the exception of the first postfire sampling in 1998 where only two individuals carrying identical haplotypes were sampled), but the haplotype composition changed. Apart from H5 (a length variant of H3), the most common haplotypes in B2 in 1997 (and overall landscapes; H1, H2, H3, and H4) have been observed in the population after the fires. In contrast, the rare and endemic haplotypes from 1997 were not found later in samples from the same landscape (see Table 3). Interestingly, in B2, a number of haplotypes, which were not encountered in 1997 and in any other landscapes, were observed in 1999, 2000, and 2004 (Table 3). These haplotypes might

Table 3 Distribution of 42 haplotypes in *Drupadia theda* populations. *N*, number of individuals sequenced per sample; *h*, total number of haplotypes per sample

	Gunung Meratus		Sungai Wain				Wanariset					Overall
	I1-00	I3-00	B1-98	B1-00	I2-98	I2-00	B2-97	B2-98	B2-99	B2-00	B2-04	
H1	1	—	7	12	12	14	2	2	1	1	3	55
H2	—	3	3	6	6	5	3	—	3	8	—	37
H3	1	2	—	—	—	—	1	—	2	4	9	19
H4	1	—	—	2	—	—	2	—	—	3	3	11
H5	5	2	—	—	—	—	4	—	—	—	—	11
H6	1	3	—	—	—	—	—	—	—	—	—	4
H7	—	2	—	—	—	—	2	—	—	—	—	4
H8	—	1	—	—	—	—	—	—	—	—	—	1
H9	—	1	—	—	—	—	—	—	—	—	—	1
H10	6	3	—	—	—	—	—	—	—	—	—	9
H11	—	1	—	—	—	—	—	—	—	—	—	1
H12	—	1	—	—	—	—	—	—	—	—	—	1
H13	—	1	—	—	—	—	—	—	—	—	—	1
H14	4	1	—	—	—	—	—	—	—	—	—	5
H15	—	1	—	—	—	—	—	—	—	—	—	1
H16	1	—	—	—	—	—	—	—	—	—	—	1
H17	1	—	—	—	—	—	—	—	—	—	—	1
H18	1	—	—	—	—	—	—	—	—	—	—	1
H19	1	—	—	—	—	—	—	—	—	—	—	1
H20	1	—	—	—	—	—	1	—	—	—	—	2
H21	—	—	1	1	—	—	—	—	—	—	—	2
H22	—	—	—	1	—	—	—	—	—	—	—	1
H23	—	—	—	1	—	—	—	—	—	—	—	1
H24	—	—	—	1	—	—	—	—	—	—	—	1
H25	—	—	—	—	1	—	—	—	—	—	—	1
H26	—	—	—	—	—	1	—	—	—	—	—	1
H27	—	—	—	—	—	—	1	—	—	—	—	1
H28	—	—	—	—	—	—	2	—	—	—	—	2
H29	—	—	—	—	—	—	1	—	—	—	—	1
H30	—	—	—	—	—	—	1	—	—	—	—	1
H31	—	—	—	—	—	—	1	—	—	—	—	1
H32	—	—	—	—	—	—	1	—	—	—	—	1
H33	—	—	—	—	—	—	1	—	—	—	—	1
H34	—	—	—	—	—	—	1	—	—	—	—	1
H35	—	—	—	—	—	—	—	—	6	2	2	10
H36	—	—	—	—	—	—	—	—	2	1	—	3
H37	—	—	—	—	—	—	—	—	1	1	—	2
H38	—	—	—	—	—	—	—	—	—	3	—	3
H39	—	—	—	—	—	—	—	—	—	1	—	1
H40	—	—	—	—	—	—	—	—	—	1	—	1
H41	—	—	—	—	—	—	—	—	—	—	4	4
H42	—	—	—	—	—	—	—	—	—	—	1	1
<i>N</i>	24	22	11	24	19	20	24	2	15	25	22	208
<i>h</i>	12	13	3	7	3	3	15	1	6	10	6	26

represent postdisturbance immigration or rare haplotypes that were not encountered in B2-97.

Tajima's *D* neutrality test revealed no significant deviation from neutral expectations in any sample (Table 4, all $P > 0.05$). *H* test revealed significantly higher values of haplotype diversity in I3-00, and in B2-97 compared to those

expected under the neutral coalescent process (Table 4). The analysis of sequence polymorphism using both Tajima's *D* test and Depaulis & Veuille's *H* test did not reveal a significant departure from neutral expectations in any of the disturbed landscapes after the 1997/1998 ENSO-induced drought and fires.

Table 4 Genetic diversity and results of neutrality tests in populations of *Drupadia theda*. *N*, number of individuals; *Hd*, haplotype diversity (Nei 1987); *S*, number of polymorphic sites; π , nucleotide diversity (Nei 1987); and *k*, mean number of pairwise nucleotide differences between individuals (Tajima 1983). For Tajima's *D* test (Tajima 1989), the value of *D* is reported. For the *H* test (Depaulis & Veuille 1998), the 95% confidence interval (CI) of simulated values of haplotype diversity based on the number of polymorphic sites under neutrality is reported, as well as the probability that $Hd_{sim} < Hd_{obs}$ (see Material and methods). Significant departures from neutral expectations are indicated in bold

	<i>N</i>	<i>Hd</i>	<i>S</i>	π	<i>k</i>	Tajima's <i>D</i>	95% CI Hd_{sim}	<i>P</i> value
I1-00	24	0.888	20	0.0072	6.638	-1.170	0.670–0.945	0.605
I3-00	22	0.948	19	0.0141	7.147	1.373	0.666–0.943	0.976
Gunung Meratus-00	46	0.923	22	0.0110	5.587	0.380	0.657–0.930	0.948
B1-98	11	0.564	2	0.0012	0.618	-0.290	0.181–0.727	0.628
I2-98	19	0.526	2	0.0011	0.561	-0.045	0.105–0.678	0.638
Sungai Wain-98	30	0.524	3	0.0011	0.568	-0.589	0.186–0.720	0.484
B1-00	24	0.703	12	0.0042	2.120	-1.168	0.539–0.905	0.155
I2-00	20	0.468	2	0.0011	0.542	-0.090	0.100–0.678	0.467
Sungai Wain-00	44	0.597	13	0.0028	1.416	-1.613	0.510–0.892	0.058
B2-97	24	0.953	15	0.0092	4.678	0.327	0.550–0.927	0.999
B2-99	15	0.810	12	0.0100	5.086	1.473	0.561–0.933	0.402
B2-00	25	0.863	15	0.0083	4.187	0.189	0.576–0.926	0.598
B2-04	22	0.788	10	0.0062	3.355	0.760	0.463–0.904	0.481

Population structure

Over all pairwise comparisons, Φ_{ST} estimates ranged from negative to 0.654 (Table 5). Within regions at time *t*, none of the pairwise Φ_{ST} were significant after sequential Bonferroni adjustment of the significance level (within Gunung Meratus in 2000, $\Phi_{ST} = 0.092$, $P = 0.018$; within Sungai Wain in 1998, $\Phi_{ST} = -0.152$, $P = 0.883$; in 2000, $\Phi_{ST} = 0.015$, $P = 0.302$). In 2000, 32.63% of the total variance was attributed to variation among the five landscapes (global $\Phi_{ST} = 0.317$, $P < 0.001$; Table 6). However, the genetic variation in 2000 did not appear significantly associated with the three regions, Gunung Meratus, Sungai Wain and Wanariset (among group genetic variance = 0.909, $P = 0.063$). After Bonferroni corrections, the Wanariset population sampled in 1997 (B2-97) did not appear significantly different from Gunung Meratus populations (I1 & I3) sampled in 2000, but the *P* values were lower than 0.01 ($\Phi_{ST} = 0.118$, $P = 0.005$, and $\Phi_{ST} = 0.109$, $P = 0.009$, respectively; Table 5). Within Wanariset, genetic divergences between years appeared significant between 2000 and 2004 ($\Phi_{ST} = 0.222$, $P = 0.001$), but not significant after Bonferroni correction between 1997 and 1999 ($\Phi_{ST} = 0.102$, $P = 0.030$), and between 1999 and 2000 ($\Phi_{ST} = 0.075$, $P = 0.062$). Also, no significant genetic divergence was observed within Sungai Wain landscapes between 1998 and 2000 (all $P > 0.05$). Interestingly, no significant divergence was observed in Wanariset between 1997 and 2004 samples ($\Phi_{ST} = 0.045$, $P = 0.091$). Amongst Wanariset (B2) and the two sampled landscapes from Sungai Wain (B1 and I2), 9.59% of the genetic variance was attributed to temporal variation among years within landscapes ($P = 0.001$; Table 6).

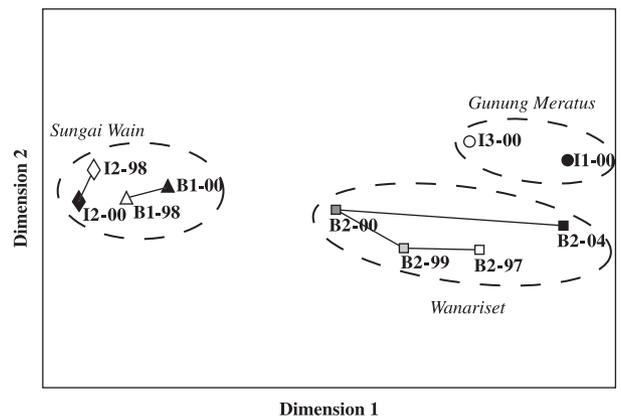


Fig. 5 Non-metric multidimensional scaling ordination of *Drupadia theda* populations based on Φ_{ST} values estimated between samples using both haplotype frequencies and molecular divergence of mtDNA sequences. Samples from the same landscape are connected by a line. Dash lines encircle regions.

The genetic divergences found amongst *D. theda* samples (with the exception of B2-98) are visualized with pairwise Φ_{ST} estimates using a two-dimensional non-metric MDS in Fig. 5. This method gives a good representation of the data with a stress value of 0.019. The MDS graphically illustrates the evolution of patterns of genetic divergences within as well as between populations. It depicts the increase of genetic divergence between B1 and I2 from 1998 to 2000, and the decline of genetic divergence between Wanariset (B2) and Sungai Wain (B1 + I2) from 1997 to 2000. It also illustrates the temporal evolution of genetic composition within Wanariset.

Table 5 Pairwise Φ_{ST} estimates between *Drupadia theda* populations (above the diagonal). Significance levels are indicated below the diagonal as *** if significant after adjustment of the significance level $\alpha = 0.05$ according to the sequential Bonferroni correction (corresponding Φ_{ST} values are indicated in bold), ** for $P < 0.01$, * for $P < 0.05$, and NS for $P > 0.05$

		Gunung Meratus		Sungai Wain				Wanariset			
		I1-00	I3-00	B1-98	B1-00	I2-98	I2-00	B2-97	B2-99	B2-00	B2-04
G.	I1-00		0.092	0.604	0.532	0.643	0.653	0.118	0.219	0.294	0.089
Meratus	I3-00	*		0.370	0.341	0.423	0.435	0.109	0.161	0.173	0.158
S. Wain	B1-98	***	***		-0.015	-0.052	-0.053	0.413	0.317	0.226	0.602
	B1-00	***	***	NS		0.003	0.015	0.334	0.255	0.148	0.513
	I2-98	***	***	NS	NS		-0.043	0.458	0.387	0.259	0.642
	I2-00	***	***	NS	NS	NS		0.470	0.401	0.275	0.654
Wanariset	B2-97	**	**	***	***	***	***		0.102	0.108	0.045
	B2-99	***	**	***	***	***	***	*		0.075	0.126
	B2-00	***	***	**	**	***	***	*			0.222
	B2-04	***	***	***	***	***	***	NS	*	***	

Table 6 Results of AMOVA for among-population differences and population structure in relation to the spatial (according to geographic regions) and temporal (according to landscapes) grouping. The analysis partitions total molecular variance into different components, whose significance was obtained by randomization after 10 000 permutations

Population structure tested	d.f.	Variance components	% of variance explained	P value
2000 only, 5 landscapes (I1, I2, I3, B1, B2), no grouping				
Among populations	4	Va 0.874	32.63	< 0.0001
Within populations	110	Vb 1.788	67.17	—
2000 only, 5 landscapes (I1, I2, I3, B1, B2), 3 groups (Gunung Meratus, Sungai Wain, Wanariset)				
Among groups	2	Va 0.909	32.06	0.063
Among populations within groups	2	Vb 0.138	4.88	0.017
Within populations	110	Vc 1.788	63.06	< 0.0001
Temporal: 3 landscapes (I2, B1, B2), 3 groups (Gunung Meratus, Sungai Wain, Wanariset)				
Among groups	2	Va 0.609	27.57	0.023
Among populations within groups	6	Vb 0.212	9.59	0.001
Within populations	153	Vc 1.389	62.84	< 0.0001

Discussion

mtDNA control region sequence polymorphism in *Drupadia theda*

Amongst 208 *Drupadia theda* individuals and the 509-bp mtDNA fragment analysed (CR+ 12S 5' end), we identified 42 haplotypes, including alignment gaps; the overall nucleotide diversity was 0.0069. When analysing the same mtDNA region as assessed by Vila & Björklund (2004), i.e. exclusively the CR 5' end prior to the homopolymer, we found a much higher nucleotide diversity in *D. theda* (325 bp; $\pi = 0.011$) than in the two nymphalid butterflies *Erebia triaria* (361 bp; $\pi = 0.0044$) and *Erebia palarica* (354 bp; $\pi = 0.0005$) (Vila & Björklund 2004). Among the 42 haplotypes, one group of haplotypes was highly divergent compared to the rest of the haplotypes (group 3; H6, H8, H9, and H11, see Fig. 2). The seven individuals carrying these divergent

haplotypes might possess an ancient mitochondrial variant preserved in the Gunung Meratus region, belong to a cryptic species, or be of hybrid origin. The analyses of the COI sequences revealed that the sequence divergence between related species (*Drupadia theda* and *Drupadia ravindra*) was nearly fivefold the divergence found between individuals carrying haplotypes of each of the groups 1 and 3. Moreover, we did not find evidence of wing pattern differences between the individuals belonging to different groups. Therefore, the lack of evidence for the occurrence of cryptic species or hybrids led us to maintain all sequenced haplotypes in the data set. Further investigations on male genitalia and other genetic markers will be needed to be more conclusive about the origin of this divergent group of haplotypes. Although the utility of the mtDNA CR for species-level phylogenies and population-level studies in insects remains controversial (Taylor *et al.* 1993; Zhang & Hewitt 1997; Caterino *et al.* 2000), our results on sequence

variability of the mtDNA control region in *D. theda* suggest that this molecular marker possesses a sufficient amount of polymorphism to conduct population genetic surveys at the spatial scales studied, in line with the results of the Vila & Björklund (2004) study on various species of Lepidoptera.

Genetic diversity vs. disturbance

Amongst *D. theda* sampled populations, the observed genetic diversity was spatially and temporally heterogeneous. Indeed, in 2000, levels of genetic diversity were relatively high in Gunung Meratus and Wanariset compared to those in Sungai Wain. Similarly, temporal changes were observed in the Wanariset population between 1997 and 2004. The Wanariset landscape B2 is located in the Wanariset Samboja Research Forest, which is not situated in a national park or reserve, but on land protected for research purposes. In 1997, the Wanariset forest presented a mosaic of forest in an advanced stage of regeneration together with primary dipterocarp forest (Slik *et al.* 2002) because it had already partially burned during the 1982/1983 ENSO event. Although numbers of *D. theda* recorded in 1997 in Wanariset per transect and per day were two to five times lower than reported in Gunung Meratus landscapes, levels of genetic diversity were comparable in these forests. In 1998, a massive population size reduction was observed in Wanariset, as the number of individuals recorded per transect and per day decreased by a factor of 10. This major bottleneck was obviously linked with the 1997/1998 ENSO-induced fires (Cleary & Grill 2004). Indeed, during the 1997/1998 winter, the Wanariset forest almost entirely (> 90%) burned (Cleary & Grill 2004). The *D. theda* that survived the fires were exclusively restricted to a c. 50-ha unburned forest fragment (namely W03 in Cleary & Grill 2004).

The Sungai Wain landscapes (B1 and I2) have been sampled in an originally pristine and protected forest (the Sungai Wain Nature Reserve) of 10 000 ha of lowland mixed dipterocarp rainforest with extensive swamp areas in low lying areas, similar to what was observed in Gunung Meratus protected forest. Despite its protected status, the forest has suffered considerable encroachment from adjacent human settlements, and fires early in 1998 destroyed about two-thirds of the pristine forest so that by mid 1998 only about 3500 ha of pristine forest remained. In 1998, both sampled landscapes B1 and I2 showed very low levels of genetic diversity combined with low number of recorded individuals. In 2000, population sizes increased while the levels of genetic diversity remained lower than in the pristine forest located in a large unburned area (I1). Because we lack temporal data from 1997, we do not have evidence that the low levels of genetic diversity originated from bottlenecks. However, because (i) the two landscapes were sampled in an originally pristine and protected forest, (ii) *D. theda* densities in Sungai Wain forest were much lower

than in Gunung Meratus pristine forest, (iii) a population crash was observed in Wanariset following 1997/1998 ENSO-induced fires, and (iv) a population size increase was observed between 1998 and 2000 in both B1 and I2, the low levels of genetic diversity observed in 1998 probably result from population bottlenecks following population crashes due to the 1997/1998 ENSO-induced disturbance.

The similar, low genetic diversity values observed in B1 and I2 further suggest that the drought associated with the fires had as negative an impact on the genetic diversity of *D. theda* as the fire itself. Indeed, while I2 was located in a small unburned isolated pristine forest, it was surrounded by burned forest. The ENSO-induced drought detrimentally impacted available water and trophic resources (Cleary & Grill 2004). Such effects are known to have caused local extinctions of several fig wasp species in an unburned forest isolate in northern Borneo due to disruption of their primary food resources (Harrison 2000). This result is further in agreement with the impact of drought on species richness (Cleary 2003; Cleary & Genner 2004; Cleary *et al.* 2004). Yet, it is still not clear if the drought affected the butterflies directly, or indirectly, through its host plants or mutualist ants. The latter was the case with most UK populations of the lycaenid *Polyommatus bellargus* that were affected by severe bottlenecks in the late 1970s when a drought caused its unique host plant (*Hippocrepis comosa*) to wilt (Harper *et al.* 2003). Nevertheless, since *D. theda* feeds on a variety of host plants, it is unlikely that the drought affected all the species host plants. Still, *D. theda* population bottlenecks could have been caused by population size reductions (or complete disappearance) of its mutualist ants of the genus *Crematogaster* (morphospecies A and B; Seufert & Fiedler 1996) which could be associated with humid soil conditions, similar to *Lasius niger*, the host ant of the lycaenid *Plebejus argus* (Seymour *et al.* 2003). Our results on genetic diversity in pre- and postdisturbed populations suggest that the ENSO-related population bottlenecks, together with genetic drift in small populations (possibly in combination with selection and variance in reproductive success), resulted in massive losses of haplotypes, leading to genetic compositions with one or two highly frequent haplotypes and a few rare haplotypes.

Interestingly, the two samples showing the highest haplotype diversity values were nonpristine; I3-00 had been logged in 1993/94, and B2-97 partially burned during the 1982/83 ENSO event. Moreover within these two samples, a significant deviation from neutrality was detected as higher haplotype diversity than observed under the hypothesis of neutrality and stable population size. These higher values may originate from sample size bias or reflect nonequilibrium conditions within these two previously disturbed forests. Interestingly, higher levels of species diversity were found in the same logged forest than the proximate pristine forest of Gunung Meratus (Cleary

2003). The high genetic diversity found in the logged forest may thus be suggestive of similar processes acting on genetic diversity and species diversity. However, because of the lack of replicates (relative to both species and logged areas), more investigations are needed to conclude that a species-genetic diversity correlation (Vellend 2003) is actually occurring in disturbed environments.

Genetic recovery following bottlenecks

A particularly interesting result from the short-term impact of droughts and fires on *D. theda* genetic diversity concerns the differences observed in genetic diversity patterns following the bottlenecks. Two years after the fires, levels of genetic diversity within Sungai Wain did not significantly increase, despite observed population expansions. On the contrary, in Wanariset, the number of individuals recorded in 1999 was similar to that in 1997, and levels of genetic diversity were reaching pre-ENSO levels. From the 40 recorded individuals in 1999, 39 were observed along the transect situated in the unburned fragment (W03 described in Cleary & Grill 2004). In 2000, high population densities were observed in Wanariset, although the recorded *D. theda* were no longer restricted to the W03 pristine fragment but were also recorded in high densities along transects in partially burned forest (e.g. W05 in Cleary & Grill (2004)).

In 1999, 2000, and 2004, new haplotypes were encountered in Wanariset, causing genetic diversity to rise. These new haplotypes most likely originated from incoming migrants because (i) none of these haplotypes were encountered in 1997 despite a large sampling effort (50% of recorded individuals were randomly sequenced), (ii) the same sampling method was used during each sampling event to avoid sampling bias (Cleary 2003), and (iii) the sampling intensity conducted in the following years was not higher than in 1997 (50%, 37.5%, and 10.4% in 1998, 1999, and 2000, respectively). The rapid recovery of the genetic diversity of *D. theda* in the Wanariset population is thereby certainly due to an influx of genetic diversity through migration.

Conversely, low levels of genetic diversity observed in Sungai Wain populations in 2000 may originate from sampling bias or the lack of incoming migrants with new haplotypes. However, the latter explanation is more likely given that similar sampling efforts were conducted in Sungai Wain and Wanariset in 2000 (11.5 and 10%, respectively, of recorded individuals were randomly sequenced). Because (i) Wanariset and Sungai Wain forests were similarly affected by the 1997/1998 ENSO-induced disturbance in terms of burning (Cleary *et al.* 2004) and (ii) small pristine fragments remained unburned in both forests (although much smaller in Wanariset), the faster recovery rate in the Wanariset population than in Sungai Wain populations may be due to their different proximities to larger

patches of unburned forest. In particular, the Wanariset forest is contiguous with the large (71 905 ha) Bukit Suharto Protected Forest Reserve to the north. Although most (93%) of this reserve also burned during the 1997/1998 ENSO event, an estimated 4800 ha escaped conflagration (Hoffmann *et al.* 1999) and probably thus served as a source of migrants for the Wanariset population. The Sungai Wain forest, on the contrary, is isolated from proximate forest by the bay of Balikpapan and Balikpapan city itself to the west and south, and by various roads and settlements, as well as twice-burned forest (including Wanariset) to the north and east (see Fig. 1). Thereby, the *D. theda* population expansion observed in I2 was not sufficient to raise genetic diversity levels in this isolated forest.

High levels of genetic diversity in documented bottlenecked populations are not uncommon and originate from sufficient allelic richness immediately after the bottlenecks (Brookes *et al.* 1997; Queney *et al.* 2000; Lefèvre *et al.* 2004) or from new haplotypes arriving via postbottleneck immigrations (Hansson *et al.* 2000; Keller *et al.* 2001; Whitehouse & Harley 2001; Colson & Hughes 2004; Takami *et al.* 2004). In the latter case, the speed at which the recovery of the genetic diversity occurs depends on the dispersal capacities of the species, and whether the immigrants come from one (propagule pool) or more (migrant pool) source populations. Our study suggests that the degree of geographic isolation of the local population relative to source populations is as well a key factor in driving the recovery of genetic variation following bottlenecks in natural populations.

Acknowledgements

All field assistants in Kalimantan are thanked for their help. We further thank W. van Ginkel for sorting all the materials and P. Kuperus, B. Voetdijk, and Y. Jimenez for laboratory assistance. We thank M. Vila for her valuable advice concerning PCR amplifications and J.A.J. Breeuwer, G. Lecaillon, and K.T.C.A. Peijnenburg who contributed to this work through insightful discussions. S. Planes, B. Emerson, and four anonymous reviewers provided helpful comments on earlier versions of the manuscript. This work was supported by grant 895.100.029 of the Netherlands Foundation for the Advancement of Tropical Research (NWO-WOTRO), within the Priority Programme 'Biodiversity in Disturbed Ecosystems'.

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