

## Comparison of rainforest butterfly assemblages across three biogeographical regions using standardized protocols

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**Abstract.** Insects, like most other organisms, are more diverse in tropical than in temperate regions, but standardized comparisons of diversity among tropical regions are rare. Disentangling the effects of ecological, evolutionary, and biogeographic factors on community diversity requires standardized protocols and long-term studies. We compared the abundance and diversity of butterflies using standardised 'Pollard walk' transect counts in the understory of closed-canopy lowland rainforests in Panama (Barro Colorado Island, BCI), Thailand (Khao Chong, KHC) and Papua New Guinea (Wanang, WAN). We observed 1792, 1797 and 3331 butterflies representing 128, 131 and 134 species during 230, 231 and 120 transects at BCI, KHC and WAN, respectively. When corrected for length and duration of transects, butterfly abundance and species richness were highest at WAN and KHC, respectively. Although high butterfly abundance at WAN did not appear to result from methodological artefacts, the biological meaning of this observation remains obscure. The WAN site appeared as floristically diverse as KHC, but supported lower butterfly diversity. This emphasizes that factors other than plant diversity, such as biogeographic history, may be crucial for explaining butterfly diversity. The KHC butterfly fauna may be unusually species rich because the site is at a biogeographic crossroads between the Indochinese and Sundaland regions. In contrast, WAN is firmly within the Australian biogeographic region and relatively low species numbers may result from island biogeographic processes. The common species at each of the three sites shared several traits: fruit and nectar feeders were equally represented, more than half of common species fed on either epiphytes or lianas as larvae, and their range in wing sizes was similar. These observations suggest that Pollard walks in different tropical rainforests target similar assemblages of common species, and, hence, represent a useful tool for long-term monitoring of rainforest butterfly assemblages.

**Key words:** Barro Colorado Island, Center for Tropical Forest Science, Lepidoptera, tropical rainforest, Panama, Papua New Guinea, Pollard walks, Smithsonian Institution Global Earth Observatories, Thailand.

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## INTRODUCTION

The structure and high species diversity that characterizes tropical forests has led many ecologists to overemphasize the similarities among biogeographically distinct forests and to downplay the differences. Although the planet's tropical forests can be categorized in a number of ways, it is clear that rainforest ecosystems have evolved independently several times, providing the opportunity for replicated study of tropical community assemblages while

exploring the unique role of taxa occurring nowhere else (Corlett & Primack, 2006). Cross-continental comparisons of rainforest communities, particularly of insects, are rare, and baseline studies need to be undertaken before anthropogenic incursions makes such studies impossible.

Habitat degradation is currently the biggest threat to tropical insects; however, the effects of climate change may soon be more pervasive (Chen *et al.*, 2009). As indicators of environmental disturbance or environmental change, butterflies are frequently used because they offer a number of logistical advantages over other potential indicator taxa (Thomas, 1991; Ghazoul, 2002; Koh & Sodhi, 2004; Gardner *et al.*, 2008). Primarily, unlike most insect groups, many butterfly species can be identified in the field, often facilitated by field guides. But while butterfly taxonomy is reasonably advanced, understanding of butterfly life histories and ecology lags behind, particularly for tropical taxa, which represent about 90% of all butterfly species. Butterflies and their larvae play important roles in ecosystem functioning, including nutrient cycling and pollination. This implies that tropical butterflies should be studied not just as potential biological indicators, but as targets of conservation in their own right (Bonebrake *et al.* 2010; Schulze *et al.*, 2010).

Unlike temperate areas, no long-term monitoring scheme for butterflies or any other insects has been established in the tropics until recently. In the absence of baseline data, the impact of climate change on butterflies and other tropical insects will be difficult to evaluate (Bonebrake *et al.*, 2010). Further, the diversity and complexity of tropical communities impedes efforts to understand them. Investigating insects in established long-term study plots may capitalize on existing floristic, phenological and climatic data, thus simplifying efforts to study tropical insects and their interactions with plants (Godfray *et al.*, 1999). The network of forest dynamics plots monitored by the Center for Tropical Forest Science (CTFS) is perhaps the most ambitious cross-continental ecological research network coordinated by a single organization (Losos & Leigh, 2004; Corlett & Primack, 2006). This network of permanent rainforest plots provides ample opportunities for long-term monitoring of insect populations and other entomological studies.

There are several methods available to monitor rainforest butterflies, each with their own limitations. Traps baited with rotting fruits are frequently used to attract adult butterflies that imbibe fermenting fruit juice (DeVries & Walla, 2001; Schulze *et al.*, 2001), and have been the subject of considerable interest in

tropical conservation biology (Schulze *et al.*, 2001, 2010). However, these traps attract only the subset of species that feed on fruits (Schulze *et al.*, 2001; Caldas & Robbins, 2003). Pollard walks, in which butterflies are counted along timed transects, were pioneered in England over 35 years ago (Pollard, 1977; Thomas, 1983), and today, butterfly monitoring with Pollard walks includes about 2000 transects scattered throughout Europe (van Swaay *et al.*, 2008). Observation counts obtained with Pollard walks are positively correlated with the abundances of individual species as estimated by mark-recapture studies (Pollard, 1979; Thomas *et al.*, 2004), and are therefore deemed to be a faithful measure of abundance. Pollard transects performed in tropical rainforests are often used as a sampling method to (a) assess local butterfly species richness while expending a minimum of effort, often censusing open habitats, because butterfly diversity tends to be higher in these habitats (e.g. Sparrow *et al.*, 1994; Caldas & Robbins, 2003; Walpole & Sheldon, 1999; Hill *et al.*, 2001; Koh & Sodhi, 2004; Tati-Subahar *et al.*, 2007); and (b) compare butterfly species richness in old-growth and disturbed forests or plantations (e.g., Hill *et al.*, 1995; Spitzer *et al.*, 1997; Ghazoul, 2002; Cleary & Genner, 2004).

Examining factors that may explain site-to-site variation in the species richness of butterfly assemblages in primary forests may illuminate changes in disturbed forests. In tropical forests, the high species diversity and reduced visibility in the understory impedes identification of butterflies “on the wing.” For this reason, tropical studies often do not include the taxonomically challenging but exceptionally diverse families Hesperiiidae and Lycaenidae (e.g., Sparrow *et al.*, 1994; Spitzer *et al.*, 1997; Ghazoul, 2002). Long-term studies with relatively high sampling effort directed at the same locality can alleviate this challenge by focusing taxonomic expertise on problem groups while amassing a suitable reference collection. Further, the use of standardized protocols at different localities is essential to understanding the dynamics of local communities and species assemblages. For this purpose, compilations of museum records and published checklists cannot replace field surveys. Locality data from these sources is unlikely to be detailed enough to assemble a credible list for a particular site, and sampling bias would most likely prevent site-specific extrapolation based on museum records. To the best of our knowledge, no study has yet attempted to compare entire understory butterfly assemblages from closed-canopy tropical rainforests among different biogeographic regions

using standardized sampling.

Several authors also emphasized that various life-history traits of tropical butterfly species, such as geographic range, host specificity, etc., may be correlated with butterfly use of particular habitats and increased vulnerability to disturbance (Bowman *et al.*, 1990; Thomas, 1991; Hill *et al.*, 1995; Spitzer *et al.*, 1997). Thus, a sound comparison of butterfly assemblages at different localities may also contrast possible differences in life-history traits of common butterfly species. Our study, performed at three CTFS permanent rainforest plots in different biogeographic regions (Neotropical, Oriental and Australian), was designed to provide a thorough description of butterfly assemblages in the understory of old-growth forests at these three localities. We compare the faunal composition, species richness, diversity and abundance of these assemblages, as well as the life-history traits of their common species, and then examine whether broad regional differences between our study sites may translate to comparable differences in butterfly species richness.

## METHODS

### Study sites

Neotropical: Barro Colorado Island (BCI) is a 1500 ha island created by the opening of the Panama Canal in 1910-1914. The 50 ha CTFS plot is located in the centre of the island, which is a biological reserve. A detailed description of the setting and of the CTFS plot may be found in Windsor (1990) and Condit (1988). Oriental: the 24 ha CTFS plot at Khao Chong (KHC) is located in protected forest of the Khao Chong Research and Conservation Promotion Station, which is part of the Khao Ban Thad Wildlife Sanctuary in southern Thailand. Australian: the third site is the newly established 50 ha CTFS plot located within the 10000 ha Wanang Conservation Area in Papua New Guinea (WAN). Vegetation at each site can be classified as semi-deciduous lowland moist forest, lowland seasonal evergreen forest, and mixed evergreen hill forest at BCI, KHC and WAN, respectively. At KHC, ridge forests are dominated by large *Dipterocarpus costatus* trees and other characteristic trees include *Shorea gratissima*, *Cynometra malaccensis*, and *Streblus ilicifolius*. Khao Chong forest phenology appears to coincide with the “general flowering” events that occur to the south of peninsular Malaysia (Center for Tropical Forest Science, 2010). Common tree species in the Wanang area include *Pometia pinnata*, *Tejmaniadendron bogorensis*, *Chisocheton ceramicus*, *Dysoxylum arborens*, *Celtis latifolia*, *Intsia bijuga*

and *Kingiodendron novogunensis*. At all CTFS plots, each tree with a diameter at breast height (DBH) of 1 cm or greater was counted, mapped, and identified to species (Center for Tropical Forest Science, 2010). The three study sites have similar latitude and elevation, but WAN has higher rainfall, BCI has a more severe dry period, and KHC has a steep slope. Tree diversity (in terms of families, genera and species of trees) is higher at KHC and WAN than at BCI (Table 1).

### Butterfly transects and identification

At each site, we used Pollard walks to calculate indices of butterfly species abundance along a linear transect that was repeatedly sampled over a given time interval (Pollard, 1977). To reduce trampling, we used concatenated Pollard transects on established trails at BCI and KHC (i.e., narrow understory paths not associated with a canopy opening). At BCI, we designated 10 transect sections each of 500 m, at KHC six transect sections each of 350 m, and at WAN, five transect sections each of 300 m (hereafter transect sections are termed “locations”; the minimum distance between locations was 200 m). To account for the steeper slope at KHC, half of the locations were sited on level terrain (hereafter ‘flatland’; 120-160 m) and half on a ridge (255-465 m). During each “walk,” one observer walked a transect section (location) at slow and constant pace in about 30 minutes while recording butterflies within 5 m of either side of the trail and to a height of 5-7 m (this was the smallest sampling unit; hereafter, one walk termed “transect”). Butterflies were either identified “on the wing” as accurately as possible (to species, genus or family); netted, identified (at BCI with a home-made field guide; at KHC from memory; at WAN with the pocket guide of Parsons, 1991) and released; or collected for processing and identification in the laboratory. At WAN, field observations of butterfly flight habits and microhabitat preferences made by experienced observers improved the ability to identify specimens in the field.

At all sites, we avoided walks on days with inclement weather (high rainfall or wind, low temperature). Locations were usually walked between 9:00 h and 15:00 h, on different days. Surveys were performed with a weighted frequency of dry/wet periods. At BCI, each location was walked three times during each of four quarterly surveys, from June 2008 to March 2010. At KHC, each transect was walked four times during each of quarterly surveys from August 2008 to November 2009. There was turnover of observers at both sites, but most transects were surveyed by six

**Table 1.** Salient characteristics of study sites. Sources: Condit, 1988; Windsor, 1990; Center for Tropical Forest Science (2010).

| Variable   | Barro Colorado Island                               | Khao Chong   | Wanang  |
|--|---|--|---|
| Biogeography   | Neotropical   | Oriental, within the transition zone between the Indochinese and Sundaland regions | Australian  |
| Coordinates  | 9.15°N, 79.85°W                                     | 7.54°N, 99.80°E  | 5.24°S, 145.08°E  |
| Elevation (m)  | 120-160   | 120-330  | 90-180  |
| Recent history   | Island isolated from rising Lake Gatun in 1910-1914 | No recent and major disturbance near the permanent plot                            | No recent and major disturbance near the permanent plot |
| Annual average rainfall (mm)                                 | 2631  | 2665   | 3440  |
| Annual average daily maximum air temperature (°C)            | 28.5  | 30.9   | 30.6  |
| Average length of the dry season (days)                      | 136   | 120  | 141   |
| Average monthly rainfall during dry season (mm)              | 64  | 82   | 88  |
| Number of tree recorded in CTFS plot with dbh $\geq$ 1cm     | 208387  | 121500   | 81971*  |
| Stems per ha in CTFS plot                                    | 4168  | 5062   | 4554*   |
| Number of tree species/genera/families recorded in CTFS plot | 298/181/59  | 593/285/82   | 553/273/83*   |
| Mean $\pm$ s.e. canopy openness (%) †                        | 3.99 $\pm$ 0.194a                                   | 6.06 $\pm$ 0.445b  | 2.02 $\pm$ 0.205c                                       |

\* Data for the first 18 ha of the 50 ha plot.

† Determined by canopy pictures and spherical densiometer, data not presented here. ANOVA,  $F_{2,76} = 20.17$ ,  $P < 0.0001$ , significant groups designated by different letters (Tukey-tests,  $P < 0.05$ ).

observers at BCI and three observers at KHC. At WAN, each location was walked biweekly from March 2008 to February 2009 by the same observer. Butterflies were identified using local collections and a variety of sources, including DeVries (1987-1997) and Warren *et al.* (2010) at BCI, Ek-Amnuay (2007) at KHC, and Parsons (1991, 1999) at WAN. Higher classification of butterflies follows Wahlberg (2006), Wahlberg *et al.* (2005, 2009) and Warren *et al.* (2009).

To examine the possibility that species at each site might be cryptic species complexes we sent legs of vouchered specimens to the Biodiversity Institute of Ontario, where cytochrome c oxidase subunit I ('DNA barcode') sequences were sequenced and evaluated using tools in the Barcode of Life Database (BOLD, see Craft *et al.*, 2010). Sequences were uploaded on the BOLD database (<http://www.boldsystems.org/>) and are publicly available (projects BCIBT, KHCBT and LEGI). Following Craft *et al.* (2010), we refrained from using subspecific names, unless DNA sequences suggested the existence of two or more species. Vouchers have been deposited at the Fairchild Museum, University of Panama (BCI), at the National Museum of Natural History in Washington

(WAN), and at the Forest Insect Museum of the Thai Department of National Parks, Wildlife and Plant Conservation (KHC). Representatives of each species will eventually be deposited in museums in the country where they were collected.

### Statistical analyses

We compared butterfly assemblages at study sites in terms of (a) subfamilial composition; (b) assemblage structure (abundance, species richness and related variables); and (c) life-history and morphological traits of the most common species (see below). Since transects were longer at BCI and were walked significantly faster than at KHC or WAN (Table 2), we standardized butterfly abundance per 500 m of transect and 30 min duration. Since rainforest butterflies appear to be particularly sensitive to unpredictable differences in climatic conditions between years (Cleary & Genner, 2004), we also compared butterfly abundances at BCI and KHC during the year 2009 (WAN data were collected in 2008 with a different frequency). We used the EstimateS 7.5 software package to calculate Morisita-Horn and Bray-Curtis

**Table 2.** Differences observed in Pollard walks at the three study sites. Unless stated, data refer to full data sets (values in brackets are for 2009). Mean are reported  $\pm$  s.e., unless otherwise indicated. For ANOVAs, different letters denote significant different means (Tukey tests,  $p < 0.05$ ).

| Variable  | BCI                            | KHC                            | WAN                            |
|---|--------------------------------|--------------------------------|--------------------------------|
| Butterfly individuals observed (data for 2009)                          | 1792 (1078)                    | 1797 (863)                     | 3331                           |
| No. species observed (data for 2009)                                    | 128 (92)                       | 131 (89)                       | 134                            |
| Sampling effort 2008-2010, person-hours (data for 2009), km walked      | 118 (81), 115                  | 70 (49), 81                    | 56, 36                         |
| Percentage of individuals identified to family/genus/species (%)        | 98.7/67.1/53.8                 | 94.6/37.8/19.4                 | 100/100/100                    |
| Percentage of species identified to species (%)                         | 80.4                           | 90.1                           | 100                            |
| Percentage of species observed to local known butterfly fauna *         | 42.6                           | 32.3                           | 68.9                           |
| Average Morisita-Horn index of similarity between pairwise locations †  | 0.859 $\pm$ 0.007 <sup>a</sup> | 0.275 $\pm$ 0.046 <sup>c</sup> | 0.767 $\pm$ 0.034 <sup>b</sup> |
| Average Bray-Curtis index of similarity between pairwise locations ††   | 0.576 $\pm$ 0.007 <sup>b</sup> | 0.212 $\pm$ 0.023 <sup>c</sup> | 0.600 $\pm$ 0.017 <sup>a</sup> |
| Average duration of one transect (min.)                                 | 32.39 $\pm$ 0.0002             | 27.28 $\pm$ 0.0003             | 28.20 $\pm$ 0.0003             |
| Average walking speed (m/min) ‡   | 15.88 $\pm$ 0.24 <sup>a</sup>  | 13.66 $\pm$ 0.25 <sup>b</sup>  | 11.02 $\pm$ 0.22 <sup>c</sup>  |
| Average corrected no. butterflies per transect of 500m and 30 min. ¶    | 7.40 $\pm$ 0.282 <sup>c</sup>  | 12.31 $\pm$ 0.729 <sup>b</sup> | 49.22 $\pm$ 2.29 <sup>a</sup>  |
| Average corrected no. butterflies per location – 15 transects in 2009 § | 109.01 $\pm$ 4.18              | 180.31 $\pm$ 20.60             | na                             |
| Coleman rarefaction for 350 individuals (no. of species $\pm$ SD)       | 77.8 $\pm$ 4.74                | 130.3 $\pm$ 1.87               | 70.5 $\pm$ 4.18                |
| Species richness estimate: Chao1 ( $\pm$ SD)                            | 171.7 $\pm$ 15.44              | 186.7 $\pm$ 18.05              | 146.1 $\pm$ 6.79               |
| Alpha log series index ( $\pm$ SD) **                                   | 39.36 $\pm$ 2.14 <sup>b</sup>  | 75.13 $\pm$ 6.22 <sup>a</sup>  | 27.99 $\pm$ 1.15 <sup>b</sup>  |
| Shannon index ( $\pm$ SD) †††   | 3.51 $\pm$ 0.02 <sup>b</sup>   | 4.49 $\pm$ 0.05 <sup>a</sup>   | 3.66 $\pm$ 0.09 <sup>b</sup>   |
| Exponent of bias-corrected Shannon entropy ***                          | 30.98 $\pm$ 2.72 <sup>b</sup>  | 64.08 $\pm$ 10.07 <sup>a</sup> | 32.27 $\pm$ 4.59 <sup>b</sup>  |
| Dominance: Berger-Parker index  | 0.220                          | 0.069                          | 0.171                          |
| Percentage of species observed as singletons (%)                        | 37.0                           | 44.0                           | 16.3                           |

\* Sources: BCI: Huntington (1932), 267 spp. but most probably ca 300 spp. (B. Srygley & Y. Basset, unpubl. data); KHC: Pinratana (1981-1988), Pinratana & Eliot (1992-1996), D.L. Lohman unpubl. data, 407 spp.; WAN: Sam (2009), 196 spp.  
 ANOVAs: †  $F_{2,12} = 203.0$ ,  $P < 0.0001$ ; ††  $F_{2,12} = 222.5$ ,  $P < 0.0001$ ; ‡  $F_{2,324} = 81.2$ ,  $P < 0.0001$ ; ¶  $F_{2,324} = 430.8$ ,  $P < 0.0001$ ; \*\*  $F_{2,12} = 74.5$ ,  $P < 0.0001$ ; †††  $F_{2,12} = 18.8$ ,  $P < 0.0004$ ; \*\*\*  $F_{2,12} = 10.53$ ,  $P < 0.0001$ .  
 §  $t$ -test:  $t = 4.32$ ,  $P < 0.001$ .

similarity indices between locations, Mao Tau species accumulation curves, Coleman rarefaction indices, Chao1 richness estimates, Alpha log series diversity indices and Shannon evenness indices, each with 50 randomizations (Colwell, 2005). The Morisita-Horn and Bray-Curtis similarity indices are biased towards common and rare species, respectively (Legendre & Legendre, 1984). We further calculated a relatively unbiased diversity metric with regard to sample size, the exponent of bias-corrected Shannon entropy (Chao & Shen, 2003a), with the software SPADE (Chao & Shen, 2003b).

Common species were defined as the top 15% in a rank-ordered list of species (most to least abundant) at each study site, with the additional proviso that “common species” had to have been collected at each location within a given site (i.e., the total number of individuals observed had to be  $\geq 10$  at BCI,  $\geq 6$  at KHC and  $\geq 5$  at WAN; Appendix S1). Our interpretation gives more weight to the results obtained with common

species as our intended monitoring scheme focuses on them. We scored the following suite of life-history traits and morphological characters for common species: adult food resources (fruits or nectar and/or puddle); known host plant species, family and growth form; host specificity (1 = restricted to one plant species; 2 = restricted to one plant genus; 3 = restricted to one plant family; 4 = broad generalist); geographic distribution (see below); use of modified habitats; membership in a known mimicry ring; larval ant attendance; wing colour patterns (system of Burd, 1994: yellow; orange; tiger; red; blue; clearwing; white and black; brown; and fore wing length (mm)). Burd’s (1994) system was followed to assess possible biases in human observers and/or emphasize different challenges in identifying visually species among sites. We do not use it to discuss the ecological significance of butterfly colour patterns (Schulze *et al.*, 2001). Butterfly traits were compiled from various sources, most notably Pinratana (1981-1988), DeVries (1987-

1997), Pinratana & Eliot (1992-1996), Parsons (1999) and Ek-Amnuay (2007). We also evaluated whether individual butterfly species preferred particular locations, times of day or habitats (flatland or ridge, KHC only) using the indicator value index (Dufrêne & Legendre, 1997). Its significance was tested for each species by Monte Carlo randomization with 1,000 permutations, performed with PC-ORD (McCune & Medford, 1999).

We adopted the system of Thomas (1991) to summarize the geographical distribution of our BCI species (1= endemic to Nicaragua, Costa Rica and Panama; 2= (i) C America, S to Panama, (ii) Nicaragua to NW South America; 3= both regions 2i and 2ii; 4= Neotropics (incl. Brazil, Bolivia and southwards). For KHC species, we modified the system of Spitzer *et al.* (1997) as follows: (1) Myanmar and Thailand excluding the peninsula; (2) zone (1), plus peninsular Thailand, Malaysia and Singapore; (3) Oriental region; (4) Australasian tropics or larger distribution. For WAN species, we modified the system of Parsons (1999) as follows: (1) New Guinea; (2) Australian; (3) Zone 2 plus Indo-Malayan (Sumatra, Java, Borneo, Philippines); (4) Australasian tropics or greater distribution. In these simple analyses, life-history and morphological traits were not corrected for phylogeny, as we wanted to

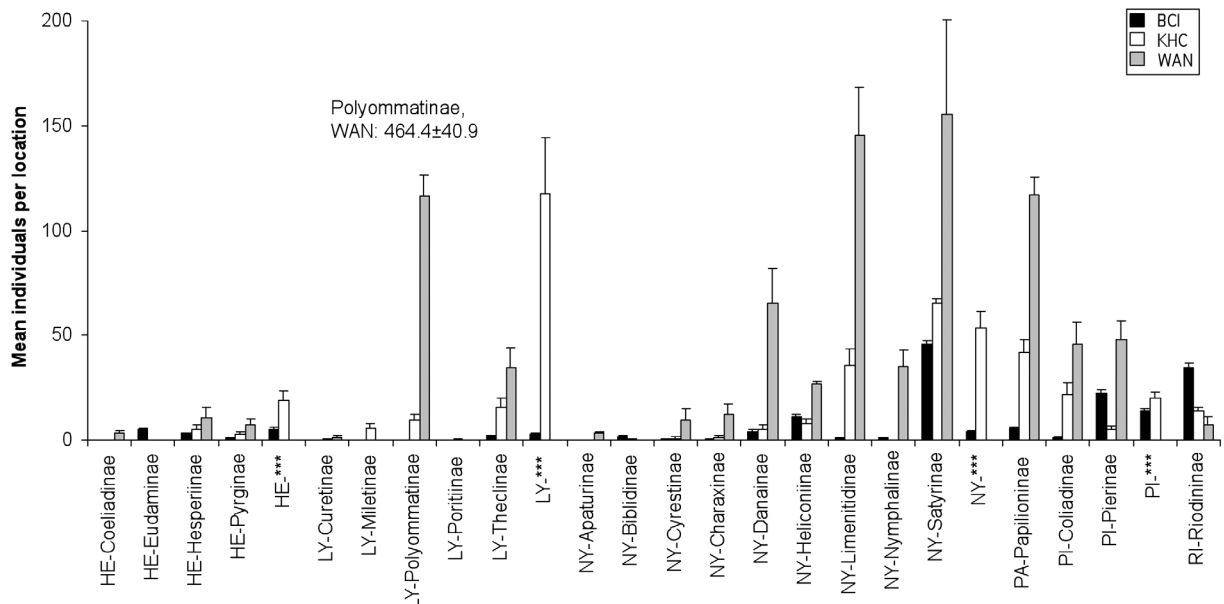
test whether these attributes may differ for a set of common butterfly species as observed with Pollard walks among study sites. The results, irrespective of phylogeny, are important to us as they could point out biases affecting the probability of detecting common species in transects (notably for wing size, wing colour pattern and cryptic life history).

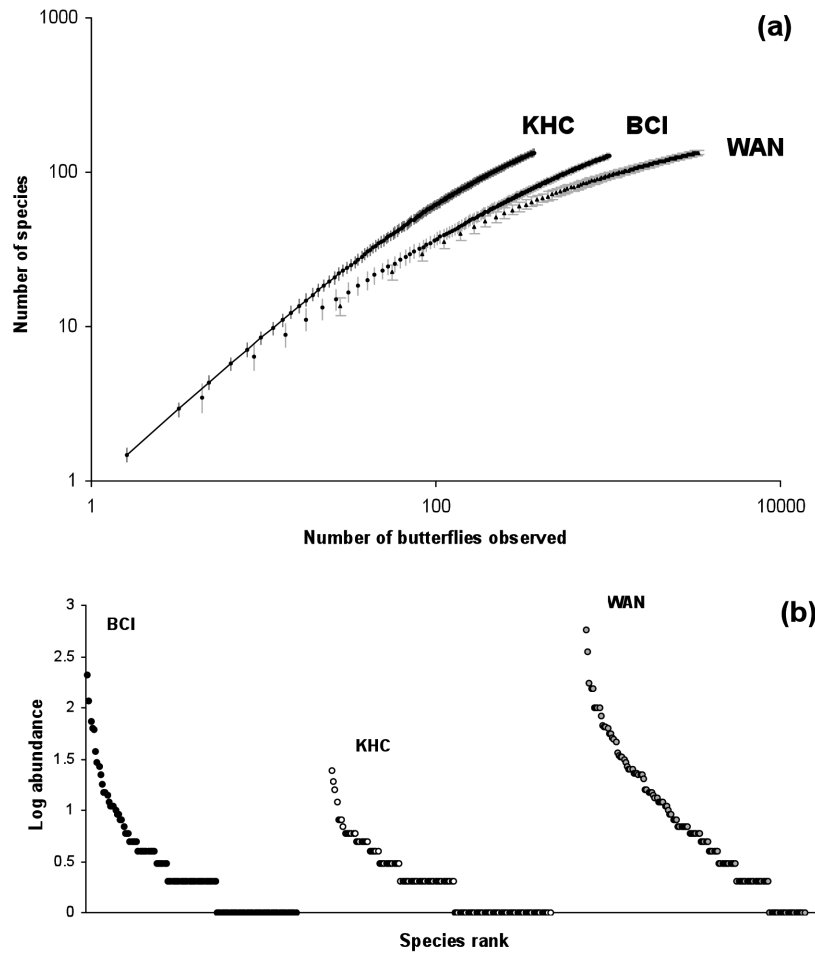
## RESULTS

### Faunal composition and structure of butterfly assemblages

We observed 1,792, 1,797 and 3,331 individual butterflies representing 128, 131 and 134 species during 7 surveys and 230 transects, 10 surveys and 230 transects, and 12 surveys and 120 transects at BCI, KHC and WAN, respectively. The more inconspicuous Hesperidae and Lycaenidae represented together 39%, 53% and 44% of observed butterfly species at BCI, KHC and WAN, respectively ( $\chi^2 = 4.97$ ,  $P = 0.083$ ). Abundance and species richness of families and subfamilies were significantly different across study sites (all  $\chi^2$  tests  $P < 0.0001$ ; Fig. 1). In particular, Eudaminae (*sensu* Warren *et al.*, 2009), Heliconiinae, Pierinae and Riodininae (BCI); Theclinae, Limenitidinae, Papilioninae and Coliadinae (KHC);

**Figure 1.** Mean number of individuals in each of the observed butterfly subfamilies at BCI (closed bars), KHC (open bars) and WAN (grey bars). Corrected mean (+ s.e.) of individuals observed per location during the whole study period. Abbreviations as follow: HE = Hesperidae; LY = Lycaenidae; NY = Nymphalidae; PA = Papilionidae; PI = Pieridae; RI = Riodinidae; \*\*\* = not assigned to subfamily. For sake of clarity, Polyommatinae at WAN were scaled by a factor 4.0.





**Figure 2.** (a) Species accumulation curve against individuals for the BCI, KHC and WAN sites. Mean ( $\pm$ SD, in grey) of 50 randomizations, logarithmic scales on both axes. (b) Species rank abundance plot at BCI (filled circles), KHC (open circles) and WAN (grey circles).

and Polyommatae, Limenitidinae, Danaeae and Papilioninae (WAN) were proportionally well represented at different study sites. The percentage of individuals that could be identified to species was significantly lower at KHC than at BCI and WAN ( $\chi^2 = 3627.9$ ,  $P < 0.0001$ ; Table 2). At WAN, all observed individuals could be identified in the field. Most of the observations at KHC that were not positively identified included unassigned Lycaenidae ( $N = 440$ ) or Nymphalidae ( $N = 202$ ), and generic identifications related to common species. The average faunal similarity between pair-wise locations was significantly different between study sites and particularly low at KHC, irrespectively of giving more weight to common or rare species (Table 2). Appendix S2 lists all species observed at the three study sites.

When corrected for length and duration of transect, butterfly abundance was about seven times

higher at WAN than at BCI, and four times higher at WAN than at KHC (Table 2). Our comparison of 15 transects at each location of BCI and KHC in 2009 also indicated a significantly higher abundance of butterflies at KHC than at BCI—nearly twice as many (Table 2). The average diversity (Alpha log series and exponent of bias-corrected Shannon entropy) and evenness (Shannon index) of locations were significantly higher and more even at KHC than at BCI or WAN (Table 2). The Chao1 estimate, the Coleman rarefaction and the steeper species accumulation curve also suggest that the species pool was richer at KHC than at BCI or WAN (Table 2, Fig. 2a). Rank species abundance plots were similar at BCI and KHC, but both plots differed from that of WAN (Kolmogorov-Smirnov two samples tests:  $D = 0.125$ ,  $P = 0.27$ ,  $D = 0.344$ ,  $P < 0.001$  and  $D = 0.410$ ,  $P < 0.001$ , respectively; Fig. 2b), because the proportion of rare

species (as estimated by the number of singletons) was lower at WAN than at other sites ( $\chi^2 = 25.03$ ,  $P < 0.0001$ ), whereas dominance was highest at BCI (Table 2). At KHC, neither butterfly abundance nor species richness differed significantly between flatland and ridge locations ( $t$ -tests,  $t = 0.05$ ,  $P = 0.96$  and  $t = 0.47$ ,  $P = 0.67$ , respectively). Butterfly abundance did not differ significantly with regard to time of day at BCI (hours tested: 9 am, 10 am, 11 am and noon; Kruskal-Wallis test,  $W = 4.78$ ,  $P = 0.189$ ), whereas it did at KHC, where abundance peaked at 11 am and was lowest at 3 pm (hours tested: 10 am, 11 am, noon, 1 pm, 2 pm and 3 pm;  $W = 20.09$ ,  $P = 0.001$ ), and at WAN, where abundance peaked at noon and was lowest at 9 am (hours tested: 9 am, 10 am, 11 am, noon, 1 pm, 2 pm;  $W = 15.44$ ,  $P = 0.031$ ).

### Life-history and morphological traits of common species

Common species included 18, 15 and 20 species, representing 78.8%, 34.4% and 73.3% of individuals identified at BCI, KHC and WAN, respectively. Appendix S1 illustrates common species at the three sites and summarizes life-history and morphological traits. Common species at each of the three sites shared several traits: fruit and nectar feeders were equally represented ( $G = 0.35$ ,  $P = 0.84$ ); more than half of common species ate either epiphytes or lianas as larvae ( $G = 0.16$ ,  $P = 0.92$ ); and common species were of similar size at the three study sites (ANOVA on forewing length,  $F = 0.22$ ,  $P = 0.80$ ). This latter trend persisted when we restricted our comparison to Nymphalidae ( $F = 0.22$ ,  $P = 0.80$ ) or Satyrinae ( $F = 1.45$ ,  $P = 0.28$ ), for which we had sufficient data. There were also notable differences between common species at our study sites. At BCI the most common species was the large dark brown Satyrinae (Nymphalidae) *Pierella luna* (Fabricius, 1793), at KHC the most common species was a large, dark brown Amathusiini (Nymphalidae: Satyrinae) *Faunis canens* (Hübner, 1826), and at WAN it was a medium-sized Polyommatinae (Lycaenidae), *Danis danis* (Cramer, 1775). Common species at BCI were more host-specific than at KHC or WAN (Kruskal-Wallis test,  $W = 8.57$ ,  $P < 0.05$ ). Common species at WAN showed higher levels of endemism than at BCI or KHC ( $W = 38.60$ ,  $P < 0.0001$ ). At KHC, the proportion of common species that were part of mimicry rings was lower than at BCI or WAN ( $G = 12.73$ ,  $P < 0.01$ ), but the proportion of common species that were attended by ants was higher than at the two other study sites ( $G = 9.20$ ,  $P < 0.01$ ). Many of the common species at KHC were duller in colour than at BCI or

WAN. When grouped into the categories of orange/brown, clearwing and other, there was a significant difference in the distribution of wing colour patterns at all study sites for common species ( $G = 13.45$ ,  $P < 0.01$ ). At BCI and KHC, most common species did not show any strong preferences for habitat, locations, or time of day (indicator values and Monte-Carlo permutations tests, Appendix S1). At BCI, only two species significantly preferred locations. At KHC, three species significantly preferred locations, habitat and time of day. At WAN, half of common species showed a significant preference for location, but only three species showed preference for flying at a particular time of day. At BCI, 28% of the common species could be found in anthropogenically modified habitats, the rest were confined to closed canopy forest. Similar data on butterfly habitat use were not available for KHC and WAN.

### DISCUSSION

Pollard walks, like other methods for surveying butterfly populations, have advantages and limitations. The main advantages are ease of implementation and the ability to survey both fruit and non-fruit feeding species. This was particularly important in our surveys since more than 80% of all common species were non-fruit feeding butterflies. In contrast, pilot studies with fruit-baited traps in Panama (in the San Lorenzo forest, 25km away from BCI), at KHC and WAN indicated either that the method had low efficiency (Panama, WAN) or that the guild of fruit-feeding butterflies was significantly less diverse at KHC because fruit feeding lineages are weakly represented (D.J. Lohman and N.E. Pierce, unpubl. data). The efficiency of fruit-baited traps and the size of the resulting sample may also be affected by variation in the availability of naturally occurring fruits (Caldas & Robbins, 2003; Walpole & Sheldon, 1999). Fruit-baited traps may be appropriate for monitoring part of local butterfly assemblages at certain rainforest locations (Schulze *et al.*, 2001, 2010), but they appear to be less suitable for comparing butterfly assemblages at locations from different biogeographical regions. Further, Hesperidae and Lycaenidae represented similar and significant proportions of total numbers of species observed at our three sites (39% to 53%). These diverse families include many camouflaged species with relatively low probability of detection, and these are typically not accounted for in Pollard walks performed in rainforests (Sparrow *et al.*, 1994; Spitzer *et al.*, 1997; Ghazoul, 2002). Our data emphasize that these species should, as far as possible, be recorded in



Pollard walks, for a more representative monitoring of rainforest butterfly assemblages.

However, there are at least four main limitations of Pollard walks when performed in rainforests. First, Pollard walks measure butterfly activity, not abundance, although the two variables are reasonably well correlated (e.g., Thomas, 1983). Second, transect counts may be affected by butterfly apparency and flight behavior (Walpole & Sheldon, 1999) and, thus, relative counts of dull versus apparent species, or smaller species, may be biased. Since the proportion of duller species appeared to be higher at KHC than at other sites, total butterfly species richness at KHC may be higher than that observed. Third, Thomas (1983) suggested that transect counts may be affected by the openness of habitats and visibility of butterflies. While this is an important consideration for comparisons between forested and open sites, this effect was unlikely to bias comparisons, because all three sites were in tall closed wet rainforests (see below). Fourth, butterflies may not be locally amenable to identification in the field with similar level of accuracy. Butterflies were more difficult to identify at KHC, partly because of a large species pool (Table 2) with many similar, dull colored species. A higher proportion of identified butterflies at KHC may have resulted in higher numbers of species observed, thus increasing differences in butterfly richness reported here between study sites. At WAN, additional field observations of butterfly flight habits and microhabitat preferences greatly improved the ability to identify species in the field. We cannot discount an observer effect (e.g., Sparrow *et al.*, 1994), however this effect was weak in multivariate analyses of common species observed in our transects (data presented elsewhere). While taxonomic training and experience was similar for observers, we expect that cultural, educational and/or training differences among observers affect their ability to identify species and may influence their propensity or reluctance to assign names to observed butterflies. We suggest that all observers in a comparative study undergo a minimum level of supervised observational training in the field by an experienced entomologist to reduce the variance among observers. The observer effect may further be reduced by randomization of observers and transect starting points, which was done in our study.

Butterfly abundance was considerably higher at WAN than at other study sites. Our corrected estimates of ca 50 butterflies per 500m of transect (strip of 10x500 m = 0.5 ha) at WAN are commensurate with estimates of 92 butterflies per 0.5 ha derived from independent mark-recapture studies of the

common species *Danis danis* and *Taenaris* spp. near the Wanang area. Further, adult survival rates and life spans of these different species at WAN also appeared similar to other tropical butterfly species (P. Vlasanek, unpubl. data). Unusually high short-term densities of butterflies may be attributed to resource concentrations for adults (Young, 1972), but unusually high long-term densities such as reported here may be related to reduced butterfly/caterpillar predation or to mutualisms with ants, which are important arthropod predators in tropical rainforests (Kaminski *et al.*, 2010; Pierce *et al.*, 2002). Since most butterfly taxa were abundant at WAN, and not just those lycaenid taxa associated with ants, this latter explanation is unlikely to be correct. The unusually high butterfly densities at WAN might also be explained by strong differences in the relative occurrence of perching vs. patrolling species (Scott, 1974), but data to test this are lacking. Since air temperature was not notably higher at WAN than at other sites and since a similar protocol was used at all sites, we conclude that differences in butterfly abundance between sites are genuine, but we cannot yet offer a convincing explanation for the observed pattern.

Differences in butterfly species richness observed at our study sites may result from a variety of causes, which may be categorized as local or regional factors. Local factors apply at the level of transects and may include forest gaps, microclimate (air temperature, wind speed and rainfall), presence of larval host plants and adult food sources, flight routes, as well as an observer effect. Analyses of potential local factors affecting our transects are all presented and discussed elsewhere. In particular, small differences in air temperature among transect days, and the occurrence of rain on days preceding a survey were important factors in explaining butterfly abundance and composition; whereas, the presence of forest gaps had only a trivial effect. All our tall closed rainforest sites had overall canopy openness <6% and there was little evidence that canopy disturbance-specialist species were prevalent at our sites (Spitzer *et al.*, 1997; DeVries & Walla, 2001).

Regional phenomena that varied among our study sites include (a) biogeographical factors, (b) recent landscape history, (c) floristics and richness of potential host-plants and (d) annual rainfall and severity of the dry season (Table 1). Our data suggest that the most species-rich site was KHC. This is confirmed by various statistics accounting for species richness and diversity (some less biased towards unequal sample size) and the larger local species pool at KHC (Table 2). This appears contrary to the views that the Neotropical region is more diverse in

butterfly species than the Oriental region and that, in particular, Panama supports a richer butterfly fauna than Thailand (Robbins, 1982, 1992). However, Robbin's (1992) comparisons do not apply specifically to forest understory in these countries. The relatively low species richness of the forest understory compared with disturbed areas is well known, even in the tropics (e.g., Spitzer *et al.*, 1997).

With regard to biogeographical factors (a), KHC (9° 40' N) is located at a biogeographic crossroads between the Indo-Burmese and Sundaland faunal regions, coinciding with a transition from aseasonal to seasonal climatic conditions (Lohman *et al.*, 2011). Immediately to the north of KHC is the Isthmus of Kra (10° 15' N), an ecotone between seasonal evergreen dipterocarp rain forest and mixed moist deciduous forests (Richards, 1996; Corbet & Hill, 1992; Hughes *et al.*, 2003). To the south of KHC is the Kangar-Pattani Line which runs west-east from Kangar, Malaysia to Pattani, Thailand (6° 40' N) and is the most widely recognized Indochinese-Sundaic biogeographic transition for plants (van Steenis, 1950; Richards, 1996). A major transition in butterfly fauna coinciding with the Kangar-Pattani Line was identified by Corbet (1941). Butterfly species recorded from the transition zone between the Isthmus of Kra and the Kangar-Pattani Line contain elements from both biogeographic regions (Ek-Amnuay, 2007). In contrast, Wanang is firmly within the Australian biogeographic region. The southern half of PNG has been part of the Australian plate for around 250 million years. The northern part, which includes Wanang was created by thrust deformation collision in the last 30 million years by the Australian Plate, which is moving north colliding with the north-western moving Pacific Plate (Hall, 2002). The relatively low species numbers at WAN is likely to result from island biogeographic processes (MacArthur & Wilson, 1967).

Recent landscape history (b) may be more relevant to BCI since the island was created by the rise in Lake Gatun in 1910-1914. The depleted butterfly fauna may be partly due to low colonization rates of certain species not able to cross the water channel (nearest forests are 0.5-3.5km distant from the island), although we do not have hard data. With regard to host plants (c), tree species are 2.0 times richer at the KHC and WAN permanent plots than at the BCI plot. We do not have similar data for herbs, lianas and epiphytes, which likely represent a large share of butterfly host-plants at our study sites (as reflected by records for our common species). Just considering tree diversity, the WAN site appeared as floristically diverse as KHC but supported fewer

butterfly species. This emphasizes that factors other than plant diversity may be crucial to explaining patterns of butterfly diversity (Hawkins & Porter, 2003). Data not presented here indicated that the effects of seasonality on butterflies (d) were low at all study sites and the wetter site (WAN) was not the most species-rich. Lepš and Spitzer (1990) also emphasized that seasonal effects are relatively low for assemblages of rainforest butterflies, as compared to similar assemblages in disturbed habitats.

Although time of day might explain temporal segregation of feeding activities by particular rainforest butterfly species (Young, 1972; but see Lepš & Spitzer, 1990), few common species showed strong preferences for flying at a particular time within the 9:00 to 15:00 h range of our transects. This suggests that the time of day during which our Pollard walks are performed in tropical forests will not significantly bias the results. Common species at each of the three sites shared several traits: fruit and nectar feeders were equally represented, more than half of common species ate either epiphytes or lianas as larvae, and their range in wing size was similar. There were few differences among our sets of common species at our study sites. Species at KHC appeared on average duller (a factor probably contributing to the low proportion of mimics at KHC), species at BCI were on average more host-specific, and species at WAN on average showed higher levels of endemism (probably related to the location of the WAN site on a large island, as opposed to the continental locations of the other sites). Although these observations remain tentative, they suggest that Pollard walks in different tropical rainforests may target similar assemblages of common species, and hence, represent a useful tool for long-term monitoring of rainforest butterfly assemblages.

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**APPENDICES S1 AND S2** (Available online. URL at [http://www.lepidopteraresearchfoundation.org/journals/44/jr1\\_44\\_17\\_28.html](http://www.lepidopteraresearchfoundation.org/journals/44/jr1_44_17_28.html))

**APPENDIX S1. Dorsal views and details of life-history traits of common butterflies species at BCI, KHC and WAN.**

**APPENDIX S2. List of all butterfly species collected at BCI, KHC and WAN.**

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