Cross-continental comparisons of butterfly assemblages in tropical rainforests: implications for biological monitoring

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Abstract. 1. Standardised transect counts of butterflies in old-growth rainforests in different biogeographical regions are lacking. Such data are needed to mitigate the influence of methodological and environmental factors within and between sites and, ultimately, to discriminate between long-term trends and short-term stochastic changes in abundance and community composition.

2. We compared butterfly assemblages using standardised Pollard Walks in the understory of closed-canopy lowland tropical rainforests across three biogeographical regions: Barro Colorado Island (BCI), Panama; Khao Chong (KHC), Thailand; and Wanang (WAN), Papua New Guinea.

3. The length and duration of transects, their spatial autocorrelation, and number of surveys per year represented important methodological factors that strongly influenced estimates of butterfly abundance. Of these, the effect of spatial autocorrelation was most difficult to mitigate across study sites.

4. Butterfly abundance and faunal composition were best explained by air temperature, elevation, rainfall, wind velocity, and human disturbance at BCI and KHC. In the absence of weather data at WAN, duration of transects and number of forest gaps accounted for most of the explained variance, which was rather low in all cases ($<33\%$).

5. Adequate monitoring of the abundance of common butterflies was achieved at the 50 ha BCI plot, with three observers walking each of 10 transects of 500 m for 30 min each, during each of four surveys per year. These data may be standardised further after removing outliers of temperature and rainfall. Practical procedures are suggested to implement global monitoring of rainforest butterflies with Pollard Walks.

Key words. Barro Colorado Island, biological monitoring, Center for Tropical Forest Science, Lepidoptera, Panama, Papua New Guinea, Pollard Walks, Thailand, tropical rainforest.

Introduction

Habitat degradation is currently the biggest threat to tropical insects; however, the effects of climate change may soon be more pervasive (Bale *et al.*, 2002; Deutsch *et al.*, 2008; Chen *et al.*, 2009). Disentangling the short-term effects of local environmental factors and seasonal variation from the long-term effects of climate change on tropical communities can be challenging because of the diversity and complexity of these communities. Biological monitoring seeks to repeat sampling over time to identify population patterns (e.g. Yoccoz *et al.*, 2001; Conrad *et al.*, 2007). Monitoring goals may be diverse, including detecting population trends of threatened, endangered, keystone, or common species. Monitoring abundant resident (common) species may be crucial for detecting the early decline of habitats (Hawking & New, 2002). In this study, we focus on monitoring the abundance of common species locally (see further discussion on this issue in Appendix S1).

Investigating insects in long-term study plots may capitalise 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 permanent forest dynamics plots monitored by the Center for Tropical Forest Science (CTFS; Losos & Leigh, 2004) provides ample opportunities for long-term monitoring of insect populations. In 2008, CTFS proposed to develop a monitoring programme, whose aims are to detect long-term changes in the abundance and composition of focal assemblages of arthropods, driven primarily by climatic cycles and changes, as opposed to short-term stochastic changes. This challenge is ideally met by comparing responses of various arthropod taxa at different rainforest sites, allowing stronger inferences regarding arthropod responses to long-term changes (i.e. a 'global' monitoring programme, Appendix S1). Annual indices consisting of various metrics are calculated for each focal assemblage. In this article, we consider principally the local abundance of all species censused together, as well as the local abundance and composition of common species (see Appendix S1 and Table S1).

Butterflies are frequently used as indicators of environmental disturbance (Thomas, 1991; Kremen, 1992; Ghazoul, 2002), and offer a number of logistical advantages over other potential indicator taxa. In particular, unlike most insect groups, many (but not all) butterfly species can often be identified in the field using field guides. Butterflies and their larvae play important roles in ecosystem functioning, including nutrient cycling and pollination (Janzen, 1987; Schowalter, 2006). 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). Unlike temperate areas, there are currently no butterfly distribution atlases and few long-term monitoring schemes for butterflies (Grøtan *et al.*, 2012) or any other insects in the tropics. Butterflies represent one of the focal taxa targeted by the CTFS monitoring programme.

There are several methods available to monitor rainforest butterflies, each with their own drawbacks (Appendix S2). In particular, passive traps baited with rotting fruits attract adult butterflies of certain species that imbibe fermenting fruit juice (DeVries & Walla, 2001). 'Pollard Walks', in which butterflies are counted along timed transects, were pioneered in England over 35 years ago (Pollard, 1977; Thomas, 1983). Today, butterfly monitoring with Pollard Walks includes about 2000 transects scattered throughout Europe (van Swaay *et al.*, 2008). Such schemes have yielded convincing data on butterfly population changes in the face of global climate change (Warren *et al.*, 2001). Observation counts obtained with Pollard Walks are positively correlated with the abundances of individual species as estimated by mark-recapture studies (Pollard, 1979; but see Harker & Shreeve, 2008), and are therefore deemed to be a faithful measure of abundance. However, as butterfly activity depends on air temperature, solar radiation, wind speed, and time of day (Douwes, 1976; Ribeiro & Freitas, 2010), these parameters should be measured during sampling and considered in subsequent statistical treatment (Kery & Plattner, 2007). Pollard Walks have been performed in tropical rainforests often with two goals in mind: (i) assessing local butterfly species richness while expending a minimum of effort, often censusing rather open habitats as butterfly diversity tends to be higher in these habitats (e.g. Sparrow *et al.*, 1994; Walpole & Sheldon, 1999; Hill *et al.*, 2001; Caldas & Robbins, 2003); and (ii) comparing butterfly species richness in old-growth and disturbed forests or plantations (e.g. Hill *et al.*, 1995; Spitzer *et al.*, 1997; Wood & Gillman, 1998; Ghazoul, 2002; Horner-Devine *et al.*, 2003; Cleary & Genner, 2004). An expert panel recommended the use of Pollard Walks for monitoring butterflies within a subset of CTFS permanent plots.

Examining factors that may strongly influence variation in the abundance or species richness of butterfly assemblages in old-growth forests may be crucial to discuss changes as they occur in disturbed forests. In tropical forests, the high species diversity and reduced visibility in the understory impede identification of butterflies 'on the wing'. For this reason, tropical studies often do not include the taxonomically challenging but exceptionally diverse families Hesperiidae and Lycaenidae (Sparrow *et al.*, 1994; Spitzer *et al.*, 1997; Ghazoul, 2002). Long-term studies with relatively high sampling effort directed at the same sites can alleviate this taxonomic challenge by focusing taxonomic expertise on problem groups while amassing a suitable reference collection. To the best of our knowledge, no study has yet attempted to compare entire understory butterfly assemblages from closed-canopy tropical rainforests in different biogeographical regions using standardised sampling.

Differences in butterfly abundance and/or species richness observed at different study sites in tropical rainforests may result from a variety of causes, which may be categorised as regional or local factors. Regional phenomena that varied notably among the study sites in this study included biogeography, recent landscape history, floristics and plant diversity, annual rainfall, and severity of the dry season (Basset *et al.*, 2011). In the present study, we focus on local factors, which apply at the level of transects within a site, and examine whether it may be possible to standardise them to reduce background noise in the data. Such factors are multiple, but we may categorise them in considering two main variables: (i) the abundance of a common butterfly species within a transect (Fig. 1) and (ii) the annual abundance of a common butterfly species within a study site (and its associated precision; Fig. S1). The main factors influenc-
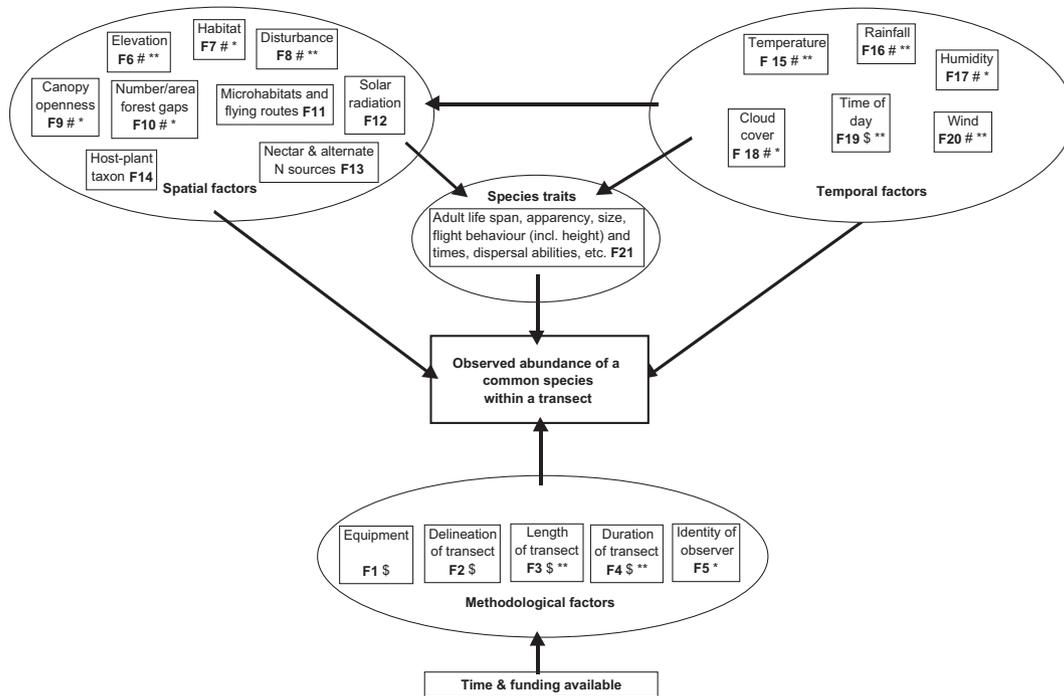
Fig. 1. Summary of main factors (boxes, F1–F21) influencing the observed abundance of an adult butterfly species within a transect in the understory of our three rainforest sites. To simplify, relationships between factors are not indicated. Key to symbols for factors: $ = easy to standardise; # = possible to standardise after removing outliers; *, ** = the effect of this factor was tested in this contribution with at least one data set (* = weak, ** = strong, see results). Factors without symbols were not tested.

ing the first variable can be categorised as methodological (factors F1–F5; often dependent on time and funding available), mostly spatial (F6–F14), mostly temporal (F15–F20), and more dependent on species traits (F21). All of these factors influence annual butterfly abundance, in addition to methodological factors dependent on the choice of transects and locations (F22–F24) and of surveys (F25, F26; Fig. S1). The same set of factor may influence the composition of common butterfly species within transects or sample units, and during the course of the year.

Our far-reaching goals (Appendix S1) are to develop adequate monitoring protocols for butterflies and other arthropods at several rainforest sites. To this end, the influence of methodological and environmental factors needs to be appreciated and, as far as possible, standardised or mitigated. However, despite the willingness of investigators to develop similar butterfly monitoring protocols at different rainforest sites, small environmental and methodological differences among sites are unavoidable and may complicate the interpretation of monitoring data. For example, there may be variation in rainfall patterns among sites, or differences in site size may result in unequal lengths and/or numbers of transects. This study seeks to evaluate the potential effect of these factors, and recommend strategies to minimise potential complications in the interpretation of monitoring data. Although we acknowledge the influence of species traits (F21) and spatial factors dependent from species behaviour and life-history (F11, F13, F14), these factors were not investigated in

this contribution. Their standardisation may be difficult or impossible within or across rainforest sites.

The specific aim of this contribution is to develop optimised protocol guidelines based on Pollard Walks for monitoring butterflies in the understory of tropical rainforests on a global scale. Our recommendations are supported by data collected at three CTFS permanent tropical rainforest plots in different biogeographical regions (Neotropical, Oriental and Australian). First, we evaluate how methodological and local environmental factors affect the collection of butterfly data in Pollard Walks and then test whether assemblages at the three study sites respond similarly to these factors. Second, we use our results to illustrate and justify methodological decisions whenever possible. Hence, our three independent study sites are used to explore the adequacy of protocols and their performance for different local faunas. Detailed descriptions of butterfly assemblages at the three sites and their differences in abundance and diversity are discussed elsewhere (Basset *et al.*, 2011).

Methods

Study sites

Neotropical: Barro Colorado Island (BCI) is a 1500 ha island created by the opening of the Panama Canal in 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 (1998). 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 10 000 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 all CTFS plots, each tree with a diameter at breast height (DBH) of 1 cm or greater has been counted, mapped, and identified to species (Center for Tropical Forest Science, 2010). Rainfall data were available from the Smithsonian Tropical Research Institute for BCI and from the Khao Chong Botanic Garden for KHC. No daily rainfall data were available for WAN. The three study sites have similar latitude and elevation, but WAN has higher rainfall, BCI has a drier dry period, and KHC has a steep slope. Tree diversity is higher at KHC and WAN than at BCI (Table 1).

Butterfly transects and identification

For sake of clarity, we hereafter use the following terms, defined as follows: study sites = the three study areas within and near the permanent plots of BCI, KHC, and WAN; locations = spatial locations within study sites where butterfly transects were performed; transects = butterfly transects ('walks') replicated at each location; surveys = butterfly transects replicated at each location and during a particular time period (see below for further details). Hence, the smallest sampling unit was one transect, which, depending on analyses, might have been pooled over surveys or locations (see below). 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). Protocols were derived from pilot studies at each study site, but their suitability for long-term monitoring is addressed in the discussion. To reduce trampling,

we used concatenated transect location ('locations') on established trails at BCI and KHC (i.e. narrow understory paths not associated with a canopy opening). At BCI, we designated 10 locations of 500 m, at KHC 6 locations of 350 m, and at WAN 5 locations of 300 m (the minimum distance between locations was 200 m; factors F3 and F23 in Figs 1 and S1). To account for the steeper slope at KHC, half of the locations were located on level terrain (hereafter 'flatland'; 120–160 m) and half on a ridge (255–465 m; F6 and F7). During each transect, one observer walked at slow and constant pace along a location in about 30 min (F4) while recording butterflies within 5 m of either side of the trail and to a height of 5–7 m (F2). 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. Prior to the start of each transect, the observer recorded air temperature (F15), relative humidity (F17), wind velocity (F20), percentage cloudiness (F18), and time of day (F19; at WAN, only the last three variables were recorded; details in Appendix S2). We use 'butterflies censused' to mean butterflies both collected and observed within a particular sampling unit.

At all sites, we avoided walks on days with inclement weather (high rainfall or wind, low temperature). Locations were usually walked between 9:00 and 15:00 hours (F19), on different days. Surveys were performed with a weighted frequency of dry/wet periods (F25, F26, see Appendix S2). At BCI, each 500 m location was walked three times during each of four annual quarterly surveys from June 2008 to March 2010. At KHC, each 350 m location was walked four times during each of four annual quarterly surveys, from August 2008 to November 2009 (F22). There was turnover of observers at both sites, but most transects were surveyed by six observers at BCI and three observers at KHC, with randomisation of observers and starting points of transect locations (F5). At WAN, each 300-m location was walked twice by the same observer during each annual monthly

Table 1. Salient characteristics of study sites.

Variable	Barro Colorado Island	Khao Chong	Wanang
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
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 ≥1cm	208 387	121 500	81 971*
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 ± SE canopy openness (%)†	3.99 ± 0.194[a]	6.06 ± 0.445[b]	2.02 ± 0.205[c]

Sources: Condit (1998); Windsor (1990); Center for Tropical Forest Science (2010).
*Data for the first 18 ha of the 50 ha plot.
†ANOVA, $F_{2,76}$ = 20.17, $P < 0.0001$, significant groups designated by different letters (Tukey tests, $P < 0.05$), see Appendix S2.

survey from March 2008 to February 2009 (F5, F22). Butterflies were identified using local collections and a variety of sources (Appendix S2).

Statistical analyses

Relative variation may be used as a simple estimator of the precision of the sampling protocol. It is calculated as the percentage of standard error (SE) relative to the mean. This approach is routinely used in economic entomology, where precision is considered as satisfying when ≤20% (e.g. Kogan & Herzog, 1983). A smaller relative variation indicates greater precision. Annual indices of butterfly abundance and their precision were calculated as the mean and SE of pooled abundance per location within a study site (details in Appendix S1). For these calculations, the independence of data points was crucial. Spatial autocorrelation (F24) can be interpreted as redundant information on data, because of the contagious effect among adjacent samples. Ignoring spatial autocorrelation leads to overly optimistic standard errors, inflated degrees of freedom, biased parameter estimates in correlation or regression analyses, and incorrect inference in statistical analyses (Dormann, 2007; details in Appendix S1). We accounted for potential spatial autocorrelation among sampling units (i.e. transects, transects pooled over surveys or locations) either by correcting the degrees of freedom in statistical tests or by including geographical distance in our multivariate analyses. Details of methods and results are given in Appendices S2 and S3. Spatial autocorrelation was not significant at BCI. Transects pooled over locations were not spatially autocorrelated at WAN and only weakly so at KHC, so

we chose this sampling unit to report faunal composition and trends in butterfly abundance at all study sites (Table S6 in Appendix S3).

As transects were longer at BCI and walked significantly faster than at KHC or WAN (Table 2), we standardised butterfly abundance per 500 m of transect and 30 min duration. We used EstimateS 8.2 software to calculate Morisita-Horn 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 100 randomisations (Colwell, 2009). To evaluate the effects of methodological factors F3, F23, F22, F25 and F26, we plotted them against the precision in butterfly abundance and/or the cumulative number of common species censused (all calculations and results detailed in Appendices S2 and S3).

We modelled the log abundance of butterflies observed in transects with a lagged-predictor model of spatial autoregression (Anselin, 1988) using the software SAM (Rangel *et al.*, 2010). The following predictor variables were used in our models: duration of transect; time of day; wind velocity; percentage cloudiness; elevation of middle point of transect location; mean canopy openness of location; number of gaps in location; sum of gap area in location; human disturbance (categorical variable accounting for how often people were encountered during transects; Table S2); air temperature; relative humidity; amount of rainfall on the day and during the 30 preceding days when the transect was performed (F4, F6, F8–10, F15–20). No rainfall data were available for WAN and thus WAN models only included the first nine of the above variables.

We used canonical partitioning to interpret spatial variance in community composition among transects (Borcard *et al.*, 2004).

Table 2. Observations in Pollard Walks at the three study sites. Mean are reported ± SE, unless otherwise indicated. For ANOVAs, different letters denote significant different means (Tukey tests, $P < 0.05$).

Variable	BCI	KHC	WAN
Butterfly individuals observed	1792	1797	3331
No. species observed	128	131	134
Sampling effort: person-hours, km walked	118, 115	70, 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 similarity between pairwise locations†	0.859 ± 0.007^a	0.275 ± 0.046^c	0.767 ± 0.034^b
Average duration of one transect (min)	32.39 ± 0.0002	27.28 ± 0.0003	28.20 ± 0.0003
Average walking speed (m min^{-1})‡	15.88 ± 0.24^a	13.66 ± 0.25^b	11.02 ± 0.22^c
Average corrected no. butterflies per transect of 500 m and 30 min§	7.40 ± 0.282^c	12.31 ± 0.729^b	49.22 ± 2.29^a
Coleman rarefaction for 350 individuals (no. of species ± SD)	77.8 ± 4.74	130.3 ± 1.87	70.5 ± 4.18
Species richness estimate: Chao1 (±SD)	171.7 ± 15.44	186.7 ± 18.05	146.1 ± 6.79
Alpha log series index (±SD)¶	39.36 ± 2.14^b	75.13 ± 6.22^a	27.99 ± 1.15^b
Shannon index (±SD)**	3.51 ± 0.02^b	4.49 ± 0.05^a	3.66 ± 0.09^b
Dominance: Berger-Parker index	0.220	0.069	0.171
Percentage of species observed as singletons (%)	37.0	44.0	16.3

*Sources: see Appendix S2.

ANOVAs: †$F_{2,12} = 203.0$, $P < 0.0001$; ‡$F_{2,324} = 81.2$, $P < 0.0001$; §$F_{2,324} = 430.8$, $P < 0.0001$; ¶$F_{2,12} = 74.5$, $P < 0.0001$;
**$F_{2,12} = 18.8$, $P < 0.0004$.

a, b, c are the results of Tukey tests, indicating significantly different means.

This method uses geographic coordinates as explanatory variables in constrained ordinations, such as canonical correspondence analysis (CCA). Environmental variables used in the ordinations included all predictor variables from our spatial autoregression models (see above), plus two other categorical values: habitat (flatland or ridge, only included for KHC, F7) and the identity of the observer (only for BCI and KHC; see Appendix S2; F5). These analyses were performed with the software CANOCO (ter Braak & Smilauer, 1998) separately for the three study sites, for either common species or for all species observed. 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 was also ≥10 at BCI, ≥6 at KHC and ≥5 at WAN). Our interpretation gives more weight to the results obtained with common species as our monitoring programme is directed towards them (Appendix S1).

Results

Overall comparisons between study sites

We observed 1792, 1797, and 3331 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. Abundance and species richness data of families and subfamilies, as well as full species lists, are discussed elsewhere (Basset *et al.*, 2011). The percentage of individuals identified to species was significantly lower at KHC (19%) than at BCI (54%) and WAN (100%; $\chi^2 = 3627.9$, $P < 0.0001$). At WAN, all individuals observed could be identified to species in the field. Most of the observations at KHC that were not positively identified included unassigned Lycaenidae ($N = 440$) or Nymphalidae ($N = 202$), and genus level identifications identical to genera of common species (Appendix S4). 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 S4). 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). The average diversity (Alpha log series), evenness (Shannon index), Chao1 estimate, and the Coleman rarefaction all suggested that the species pool was richer at KHC than at BCI or WAN (Table 2; Basset *et al.*, 2011). While we summarise below our main results, the relative importance of each factor affecting butterfly assemblages is detailed in Appendix S3.

Methodological factors affecting butterfly assemblages

Transect length had a trivial but important effect of accumulating observations with distance, similar to that of the duration of transect, which influenced significantly butterfly abundance at KHC and WAN (see below). Additionally, a plot of transect length against precision in butterfly abundance suggested that transect length was adequate at BCI or WAN, but would need to be increased at KHC (Fig. S2). The identity of observer influenced weakly but significantly the composition of butterfly assemblages (see below). Transect replication might have been easily standardised to three replicates, preferably performed by different observers (Fig. S3). The number of locations might have been standardised across study sites by considering the minimum number of locations to census common butterfly species with good precision in abundance (BCI: > 7, KHC: > 6, WAN: 5; Fig. 2). As already explained in Methods, spatial autocorrelation was severe at KHC, intermediate at WAN, and low at BCI (Table S6). The timing of surveys within a year appeared not to be so critical, as long as censuses were performed during both the driest and wettest periods of the year (Fig. S4). A minimum of four surveys performed per year were adequate at WAN, but six or twelve surveys per year may improve precision in butterfly abundance, providing that time and funding are available (Fig. S5).
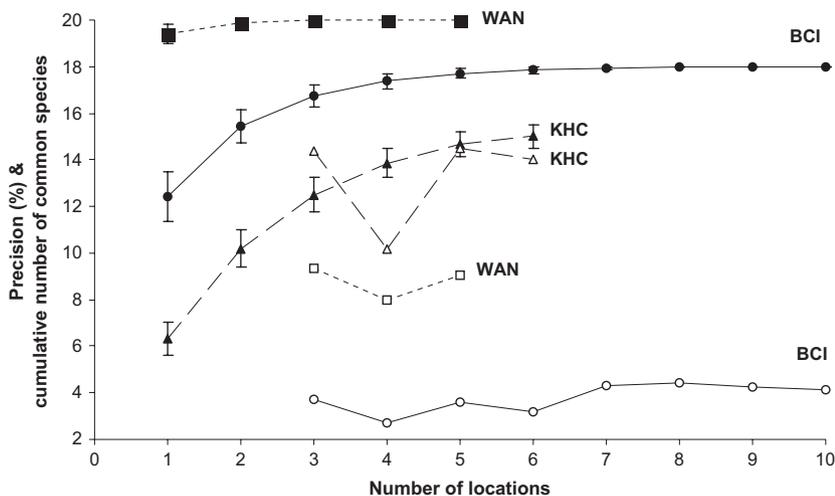
Fig. 2. Plot of the number of locations at the three study sites (BCI: circles and straight lines; KHC: triangles and broken lines; and WAN: squares and dotted lines) against precision in abundance for all butterflies censused during one year (open markers) and cumulative mean (and *SD*) randomised number of common species (filled markers). Note that smaller precision is better and that at least three locations are needed to calculate a mean of annual butterfly abundance and associated precision.

Environmental factors affecting butterfly abundance

At BCI, 24% of the variance in log butterfly abundance in transects could be explained by a combination of air temperature, elevation, human disturbance, and wind velocity after accounting for spatial autocorrelation (Table S3). At KHC, our best model explained 20% of the variance and included air temperature, duration of transect, time of day, and total rainfall for the 30 preceding days as significant variables (Table S3). 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). In the absence of temperature and rainfall data at WAN, our best model only explained 8% of variance and included duration of transect as the only significant variable (Table S3). However, at WAN, mean abundance of butterflies per transect was significantly lower in the dry season than in the wet season ($t = -5.82$, $P < 0.001$), whereas it did not differ significantly at BCI and KHC ($t = 1.81$, $P = 0.074$ and $t = -1.06$, $P = 0.29$ respectively). At BCI, air temperatures also tended to be lower during the dry season than during the wet season, but not significantly so ($t = -0.848$, $P = 0.37$). Thus, trends for higher abundance of butterflies during the relatively cool dry season at BCI explained the negative coefficient of temperature in our model of butterfly abundance for BCI (Table S3). 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; $W = 15.44$, $P = 0.031$).

Environmental factors affecting the composition of butterfly assemblages

Variance in the composition of common butterfly species was poorly explained by our weather and data collection variables. Overall, 18%, 33%, and 20% of variance was explained at BCI, KHC, and WAN, respectively, accounting for spatially and non-spatially structured environmental variance, as well as spatially structured variance not explained by environmental variables (Table S4). Of the small fraction of variance explained by the environmental variables included in the CCAs, the canonical axes were best explained by wind, total rainfall during the 30 preceding days, time of day, and the identity of the observer at BCI; by elevation, day rainfall, gap area, and high human disturbance at KHC; and by the number of gaps, duration of transects, gap area, and elevation at WAN (Fig. 3; Table S4). For the common species dataset, the fraction of variance that was purely spatial or spatially related to environmental variables was highest at WAN and lowest at BCI (Table S4). At all study sites, butterfly faunal composition was significantly influenced by season (wet or dry), but the variance explained was relatively low (CCA with only a seasonal categorical variable; BCI:

$F_{4,199} = 4.93$, $P = 0.005$, 2.4% of total variance explained; KHC: $F_{4,199} = 5.55$, $P = 0.005$, 6.1% of total variance explained; WAN: $F_{4,199} = 8.32$, $P = 0.005$, 6.5% of total variance explained). The fraction of variance in the faunal composition of all butterfly species that was explained was lower (9%, 16%, and 11% for BCI, KHC, and WAN respectively). Significant variables included two observer effects, relative humidity, and percentage cloudiness at BCI; elevation, total rainfall during the 30 preceding days, canopy openness, and time of day at KHC; and canopy openness, gap area, percentage cloudiness, and elevation at WAN (Table S5).

Discussion

Limitations of pollard walks in tropical rainforests

Pollard Walks, like other methods for monitoring butterfly populations, have advantages and limitations. The main advantages are ease of implementation, the ability to survey both non-fruit and fruit feeding species (the former representing > 80% of all common species in our transects, Appendix S4), and valuable sampling of multiple habitats by walking relatively long distances. In contrast to Pollard Walks performed in open temperate habitats, monitoring butterfly populations in tropical rainforests with Pollard Walks is challenging. In tropical rainforests, butterfly populations are rather low, visibility is reduced, species pools are large, and cryptic species often common. Hence, a smaller proportion of species are likely to be identified in the field. We list in Appendix S5 procedures that may help increase the rate of species identification. More specifically, at least four potential drawbacks may limit the applicability of Pollard Walks in tropical rainforests.

1 It represents a measure of butterfly activity, not abundance. Although activity and abundance are reasonably well correlated at higher latitudes (e.g. Thomas, 1983), this could represent a considerable bias in tropical environments with high temperature and rapidly changing cloud cover. Our regressions modelling butterfly abundance at BCI and KHC suggest that while cloud cover and relative humidity may explain little variance, air temperature and, at BCI, wind velocity, may indeed be important in this regard (see also Ribeiro & Freitas, 2010). Thus, with global air temperature rising rapidly (Parmesan & Yohe, 2003), any rise in butterfly abundance as recorded by Pollard Walks at tropical sites would need to be substantiated by independent measurements, such as mark-recapture studies or distance sampling (Yoccoz *et al.*, 2001) for some of the most common species.

2 Thomas (1983) suggested that transect counts may be affected by the openness of habitats and visibility of butterflies. Alternatively, forest gaps may provide distinct microhabitats frequented by edge and canopy species. While this is an important consideration for comparisons between forested and open sites, this effect appeared to be low in our study, because all

Fig. 3. Multivariate analyses for common butterfly species in transects at BCI (a, b), KHC (c, d), and WAN (e, f). Biplot of species (small filled circles) and transects (large open circles) in the first two axes of the Detrended Correspondence Analysis (a, c, e). Triplot of species (some identified by codes), transects, and environmental variables (only major ones identified) in the first two axes of the Canonical Correspondence Analysis (b, d, f). See methods for description of variables and Appendix S4 for species codes.

three sites were in tall closed wet tropical rainforests with overall canopy openness <6%. None of our models of butterfly abundance was significantly influ-enced by canopy openness or forest gaps. Still, forest gaps explained a small fraction of the variance in but-terfly composition at KHC and WAN.

3 Understory transects may miss a significant proportion of canopy disturbance-specialists (DeVries & Walla, 2001), but as our protocols aimed at recording changes in the abundance or composition of common understory species, this was not a problem.

4 Transect counts may be affected by butterfly apparency and flight behaviour (Walpole & Sheldon, 1999) and, thus, relative counts of dull versus apparent species, or smaller species, may be biased. Such bias does not affect the ability to assess change for a single species over time, but may influence inter-species comparisons (Dennis *et al.*, 2006). Consequently, some butterflies may not be locally amenable to identification in the field with similar levels of accuracy. Butterflies were more difficult to identify at KHC, partly because of a large species pool and many similar, dull coloured species (Basset *et al.*, 2011). At WAN, additional field observations of butterfly flight habits and micro-habitat 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 censused in our transects. This observation is crucial for butterfly monitoring programmes using Pollard Walks and targeting common species in tropical rainforests. All our assistants had been trained before the start of this project, so we were not comparing seasoned observers with novice observers. However, cultural or educational differences among observers may also affect their ability to identify species and may influence their propensity or reluctance to assign names to observed butterflies. Some cultures have more indigenous names for animals (including insects) than others, because these animals are important for survival as food, medicine, etc. Hence, those people are more likely to be able to recognise subtle differences and assign names to species (Diamond, 1989). In some cultures where there is uncertainty, there may be a tendency to err on the side of caution, that is not assigning a name. Whereas training experience may be standardised across observers and study sites, cultural component is more difficult to factor out. All observers should undergo a minimum level of training in the field by an experienced entomologist to reduce the variance among observers, and use locally adapted identification guides. In addition to improved training, the observer effect may be reduced by randomisation of observers and starting points of transect locations, as was done at all our sites.

At KHC and WAN, our transect data were significantly spatially autocorrelated, whereas this was not the case at BCI. The proportion of explained variance in faunal composition that was spatially structured was also higher at KHC and WAN than at BCI. Spatial autocorrelation at KHC probably resulted from the smaller study area, fewer, and shorter transect locations, and clumped locations along an elevation gradient. Our specific recommendations for reducing spatial autocorrelation and a straightforward interpretation of transect counts in tropical rainforests would be ideally to implement transect locations ≥500 m over a large (≥50 ha) and topographically homogeneous study area. Overall, the BCI protocol appeared superior over KHC or WAN protocols (results of analyses for factors F3–F5 and F22–F26) and generated adequate precision in abundance for common butterfly species (Table S1). The BCI protocol (three observers walking each of 10 locations of 500 m for 30 min each, during each of four surveys per year) may thus be recommended as a starting point for monitoring the abundance of common butterfly species in the understory of tropical rainforests. This protocol may further be refined in areas of high butterfly abundance, such as at WAN.

Environmental factors affecting butterfly assemblages in tropical rainforests

Butterfly abundance was considerably higher at WAN than at other study sites. Our corrected estimates of ca. 50 butterflies per 500 m of transect (strip of 10×500 m = 0.5 ha) at WAN are commensurate with independent mark-recapture studies of common species *Danis danis* and *Taenaris* spp. near the Wanang area, which estimate 92 butterflies per 0.5 ha (P. Vlasanek unpubl. data). We cannot yet offer convincing explanations for the high butterfly abundance at WAN (Basset *et al.*, 2011). The variance explained by our best models to explain abundance and faunal composition of common species was low at all study sites (< 33%). The large proportion of unexplained variance may originate from many factors, including the presence of host plants, mud puddles, fruits, floral/extrafloral nectar, other nitrogen sources, dispersal abilities, particular flying routes ('understory openness'), etc. This emphasises the difficulty of predicting the composition of species-rich assemblages including representatives with varied life histories. This was confirmed by the low variance of models that included all species observed—even at WAN where sample size was higher than at other sites. This contrasts with the often large differences in faunal composition of tropical butterflies observed within gradients of anthropogenic disturbance (e.g. Hill *et al.*, 1995; Spitzer *et al.*, 1997).

The environmental variables that explained at least some of the variance in our abundance and faunistic models at BCI and KHC were air temperature, elevation, rainfall, wind velocity (only at BCI), and human disturbance. In the absence of temperature and rainfall data, WAN models explained little. Air temperature and solar radiation greatly influence butterfly activity, whereas wind speed and time of day are less important (Douwes, 1976). Elevation may interact in aligning flying routes of butterflies into streams and watersheds (Young, 1972). Wind and rainfall account for seasonal effects, which were relatively low at all study sites. Human disturbance may perturb resting spots and/or induce trampling of host plants (Comita *et al.*, 2009). The influence of time of day, which may explain segregation in feeding activities of some rainforest species (Young, 1972), was not well marked, as reported in other studies (Lepš & Spitzer, 1990). In Europe, Pollard Walks are performed only on days when temperature, rainfall, and wind are within accepted ranges (van Swaay *et al.*, 2008). Our data suggest that optimum monitoring of common butterflies in closed canopy tropical

rainforests may be achieved by removing outliers of elevation, rainfall, and human disturbance within study sites, and that air temperature should be imperatively recorded for a sound interpretation of the data. Further standardisation may be necessary for comparing sites at rather different latitudes and elevations.

Conclusions

Pollard Walks are a convenient and expedient method for evaluating assemblages of common butterflies in old-growth forests, and we have shown that the method can be successfully extended to the evaluation of such assemblages across different biogeographical regions as long as the main methodological factors can be standardised. Methods for reducing observer bias are suggested as well as practical procedures to implement global monitoring of tropical rainforest butterflies with Pollard Walks (Appendix S5). We hope to use our data as a baseline for identifying habitat-specific species and their potential vulnerability to anthropogenic disturbance (Ghazoul, 2002) and for assessing the effects of global climatic changes on tropical insect communities (Bale *et al.*, 2002). Although warming in the tropics may be relatively small in magnitude, it is likely to have deleterious consequences for tropical insects, as they are relatively sensitive to temperature change and are currently living very close to their optimal temperature (Deutsch *et al.*, 2008). Although our data do not constitute evidence of the effect of global climate change on tropical butterflies, they show that (i) tropical butterflies are sensitive to temperature and as such are good candidates for a global monitoring scheme; and (ii) common butterfly species may be censused with adequate precision in tropical rainforests, provided that monitoring protocols consider the recommendations presented here.

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Supporting information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/j.1752-4598.2012.00205.x:

Appendix S1. Monitoring arthropods at CTFS permanent plots.
Appendix S2. Supplementary methods.
Appendix S3. Supplementary results.
Appendix S4. List of common butterfly species.
Appendix S5. Suggested procedures for monitoring butterflies inside tall closed tropical rainforests.

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