

# Population genetics of ecological communities with DNA barcodes: An example from New Guinea Lepidoptera

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**Comparative population genetics of ecological guilds can reveal generalities in patterns of differentiation bearing on hypotheses regarding the origin and maintenance of community diversity. Contradictory estimates of host specificity and beta diversity in tropical Lepidoptera (moths and butterflies) from New Guinea and the Americas have sparked debate on the role of host-associated divergence and geographic isolation in explaining latitudinal diversity gradients. We sampled haplotypes of mitochondrial cytochrome *c* oxidase I from 28 Lepidoptera species and 1,359 individuals across four host plant genera and eight sites in New Guinea to estimate population divergence in relation to host specificity and geography. Analyses of molecular variance and haplotype networks indicate varying patterns of genetic structure among ecologically similar sympatric species. One-quarter lacked evidence of isolation by distance or host-associated differentiation, whereas 21% exhibited both. Fourteen percent of the species exhibited host-associated differentiation without geographic isolation, 18% showed the opposite, and 21% were equivocal, insofar as analyses of molecular variance and haplotype networks yielded incongruent patterns. Variation in dietary breadth among community members suggests that speciation by specialization is an important, but not universal, mechanism for diversification of tropical Lepidoptera. Geographically widespread haplotypes challenge predictions of vicariance biogeography. Dispersal is important, and Lepidoptera communities appear to be highly dynamic according to the various phylogeographic histories of component species. Population genetic comparisons among herbivores of major tropical and temperate regions are needed to test predictions of ecological theory and evaluate global patterns of biodiversity.**

community ecology | DNA barcoding | phylogeography | plant–insect interactions | speciation

The distribution of tropical insect diversity is poorly understood due to incomplete taxonomic knowledge that hinders identification of species and geographic ranges. Recent estimates of beta diversity, or change in species diversity among locations, in New Guinea lowland rain forests (1) challenged assumptions about geographic isolation and ecological specialization of tropical insect herbivores. Experimental rearing of caterpillars from widespread and locally endemic vegetation indicated low host specificity compared with predictions of ecological theory on species coexistence. If competition among species were more intense in diverse communities (2), then we might expect herbivores to exhibit greater dietary specialization in the tropics (3, 4); however, host specificity in New Guinea is similar to that of a temperate herbivore community (5). Dyer et al. (6) found quite the opposite in a broader survey of caterpillars across a latitudinal gradient in the Americas. A possible explanation for the low host specificity reported by Novotny et al. (7) would be the unrecognized, but common, occurrence of host-associated cryptic species; for example, Hebert et al. (8) identified cryptic and host-specialized species in a single polyphagous and widespread neotropical butterfly. However, “DNA barcoding,” an efficient means of species diagnosis, need not necessarily reveal cryptic

specialists, and may in fact confirm polyphagy (9). Population genetic analyses of entire herbivorous insect communities are needed to understand the extent of host-associated differentiation (10); no such studies have yet been reported in the tropics, however.

Population genetic analyses also can test biogeographic hypotheses of vicariance, including explicit phylogeographic predictions in response to geologic events (11). The low beta diversity of New Guinea Lepidoptera (1) challenges a prevailing view of New Guinea biogeography (12) that plate tectonics and vicariance were integral to the origin of a diverse, endemic fauna (13). However, the broad distributions of lowland rainforest Lepidoptera species suggest that dispersal plays a more significant role than vicariance in shaping the genetic structure of these populations. Underestimation of beta diversity also could arise from taxonomic failure to recognize geographically isolated cryptic species. Molecular genetic analysis can reveal such cryptic diversity and assess the extent of migration among regional populations.

We sampled mitochondrial DNA haplotypes of 28 common Lepidoptera species (and species complexes) from multiple locations and host plant species to estimate population divergence in relation to geography and host specificity (Fig. 1). Population genetic analysis of “DNA barcodes” provides opportunities to identify overall patterns of dietary specialization and phylogeography. Diet and distributional variation among ecologically similar herbivores suggest that speciation through specialization (14) is important but not universal, and that community composition is highly dynamic in recent evolutionary time.

## Results

Mitochondrial DNA haplotypes comprising 575 bp of the cytochrome *c* oxidase I (COI) gene were sampled from 1,359 individuals and 28 Lepidoptera morphospecies, hereinafter referred to as species (GenBank accession nos. FJ499817–FJ501172). We identified 344 unique haplotypes, with each species having between 1 and 25 haplotypes (Table S1). Twenty-seven of 28 species were monophyletic and well supported according to Bayesian analysis of unique haplotypes (Fig. S1). These results confirm that parataxonomists were generally accurate in circumscribing lineages.

More than one-third of the taxa (11 of 28) showed deep intraspecific sequence divergence [Kimura 2-parameter (K2P)

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. FJ499817–FJ501172, GQ479199, GQ479200, GQ844279, and GQ844281–GQ844288).

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distance  $\geq 2\%$ ], hereinafter referred to as cryptic lineage diversity (see SI Phylogenetic Analysis). The number of cryptic lineages per species varied from two to five, with seven species having more than two cryptic lineages (Fig. S1). Four taxa exhibiting cryptic lineage diversity also were considered species complexes based on morphology, and analyses of molecular variance were limited to the largest available set of haplotypes  $< 2\%$  divergent under K2P in each case. In such cases, population genetic units referred to as species are not fully concordant with named species (see *SI Moth Taxonomy* for notes on species complexes).

No universal pattern of population genetic structure was seen among 28 Lepidoptera species in lowland New Guinea according to haplotype networks and analysis of molecular variance (Fig. 2 and Tables S2 and S3). The majority of species exhibited population differentiation associated with host plants, geography, or both. One-quarter of species showed no differentiation, and results were equivocal for the remainder. Representative haplotype networks coded for geographic distribution and host association are shown in Fig. 3. Haplotype networks revealed geographically associated genetic structure in 11 of 28 species (Table S3). Five of these cases involved genetic structure along an east-west axis through the Sepik and Ramu river basins (Fig. 1), but no case identified the Sepik as a barrier to dispersal between northern and southern Lepidoptera populations. Geographically associated cryptic lineages were observed in 6 of the 11 species.

Population genetic analyses are summarized in Table S2 for all species but one, in which all individuals carried the same haplotype regardless of site or host. Ecologically similar species showed substantial variation in patterns of spatial genetic structure,

ranging from apparent panmixia to strong isolation by distance. In four species, most of the genetic variance was distributed among locations, suggesting a high degree of spatial structure. In two of these, the extent of differentiation was much higher among populations than within populations; in the other two, it was only slightly higher among populations. Genetic diversity of local populations also varied among sites (Table S4). Geographic  $\Phi_{ST}$ , ranging from  $-0.051$  to  $0.870$ , was significant in 20 species (Table S2). Nucleotide diversity and numbers of haplotypes at each locality are reported in Tables S5 and S6, respectively. The percentage of significant pairwise  $F_{ST}$  comparisons within species ranged from 0 to 90% among species, and was  $\geq 50\%$  in 11 species (Table S2). Mantel tests of isolation by distance revealed a statistically significant correlation between geographic and genetic distance in 2 of 27 species, "*Coeliorhycidia nitidalis*" and *Unadophanes trissomita*, with coefficients of  $0.76$  ( $P = 0.050$ ) and  $0.48$  ( $P = 0.025$ ), respectively.

Haplotype networks revealed host-associated population genetic structure in nine species, whereas  $\Phi_{ST}$  was significant in 15 of 27 species (Tables S2 and S3). Six species appeared to be cryptic host specialists similar to the pattern reported by Hebert et al. (8); for example, *Rhodoneura aurata* included monophagous cryptic lineages specializing on either *Macaranga quadriglandulosa* Warb. or *Ficus concephalifolia* Ridley. In contrast, five oligophagous specialists showed no genetic differentiation associated with host species. The majority of genetic variance in all Lepidoptera species was distributed among individuals reared from the same host species, with less genetic differentiation among individuals feeding on different host species. Fifteen Lepidoptera species exhibited low but significant genetic differentiation among host species, and three of these exhibited genetic

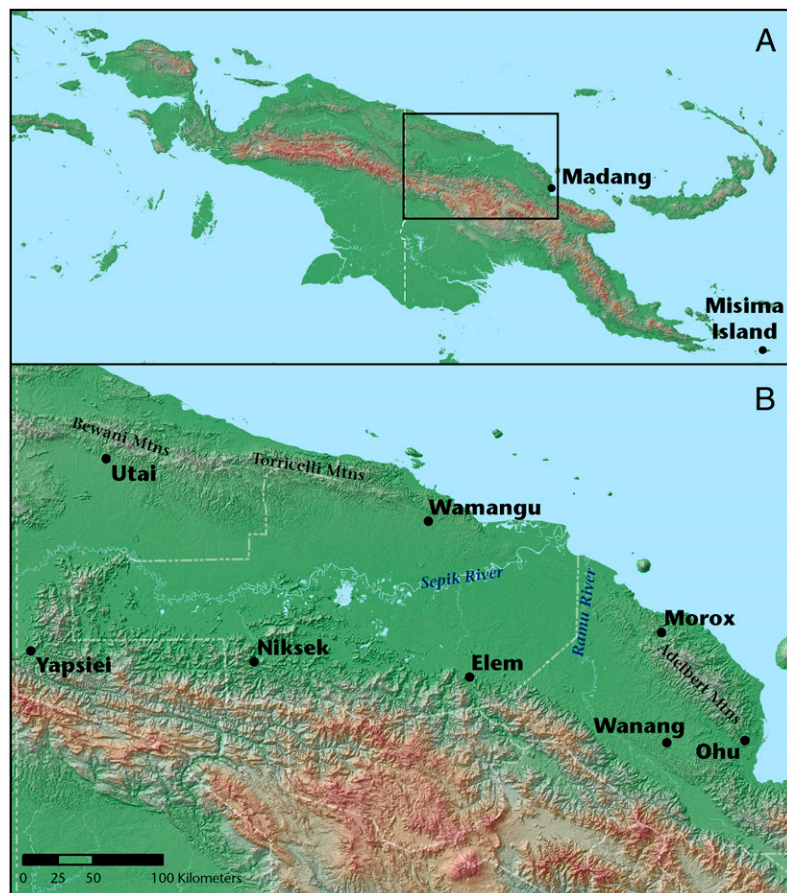


Fig. 1. (A) The island of New Guinea, with a rectangle marking the study area in the northern lowlands near the coastal town of Madang, Papua New Guinea. (B) Eight sites located in the Sepik-Ramu river basin that were the focus of Lepidoptera sampling by Novotny et al. (7).

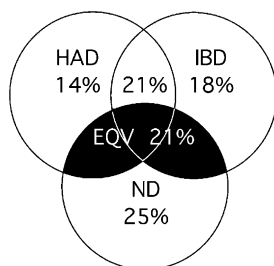
structure among host clades; for example, 30.35% of the genetic variance in *Meekiaria* sp. complex was distributed among *Psychotria* host clades. Host-associated  $\Phi_{ST}$  values ranged from  $-0.145$  to  $0.438$ , and the percentage of significant pairwise  $F_{ST}$  comparisons ranged from 0 to 66.7%. Mantel tests of Lepidoptera population genetic isolation by host plant phylogenetic distance showed host clade-associated differentiation in two species, *Arctomis intacta* complex and *Talanga deliciosa*, with correlation coefficients of 0.49 ( $P = 0.007$ ) and 0.74 ( $P = 0.027$ ), respectively.

## Discussion

Population genetic analysis of ecological communities with COI sequences extends the value of the DNA barcode beyond the realm of species identification. Whereas barcoding for taxonomic purposes is often limited by economic constraints to a few individuals per species, larger samples provide population genetic information applicable to a range of ecological and historical questions. We compared mitochondrial DNA haplotype networks and analyses of molecular variation from numerous species in the same community to assess the generality of host-associated population structure and the degree of isolation by geographic distance in tropical Lepidoptera. We examined a relatively large number of sympatric species with sufficient sampling to identify common phylogeographic patterns and host-associated population structure at the community level. Broad sampling, not influenced by a priori expectations on the extent of genetic differentiation or cryptic species, is essential to detect overall tendencies in ecological communities.

Insofar as mtDNA haplotype diversity reflects historical population dynamics, members of lowland rainforest Lepidoptera communities in New Guinea do not share identical histories, but differ by degrees of dietary specialization and geographic isolation (Table S2). Population genetic differentiation was substantial in approximately half of the species sampled. We assigned species to one of five groups based on comparisons of haplotype networks and analyses of molecular variation (Fig. 2). Four species exhibited cryptic dietary specialization throughout the geographic range of broadly sympatric host plants, whereas five species showed evidence of isolation by distance while ranging in diet from oligophagy to polyphagy. Six species exhibited both geographic and host-associated population subdivision, but seven species demonstrated neither. The status of six species was uncertain insofar as analyses of molecular variance contradicted qualitative inferences from haplotype networks. Haplotype networks of selected species (Fig. 3) serve to illustrate unequivocal cases. Here we discuss each pattern in detail and explore its implications for evolutionary ecological processes affecting tropical insect herbivore communities.

**Dietary Specialization.** Cryptic dietary specialization in the absence of geographic population subdivision is illustrated by *Addaea pusilla* (Fig. 3A and B), in which two broadly sympatric lineages exhibited



**Fig. 2.** Percentage of 28 Lepidoptera species exhibiting host-associated differentiation (HAD), isolation by distance (IBD), no differentiation (ND), and equivocal population genetic patterns (EQV) where analyses of molecular variance conflicted with haplotype networks.

host-associated genetic differentiation. Although the lineages shared some host species in common, there were also exclusive associations with different hosts in sympatry. At Oahu, for example, one lineage was reared from *Macaranga quadriglandulosa*, whereas the other was reared from *M. clavata*, *M. fallacina*, and *M. dulcis*. A similar pattern was observed in *Meekiaria purpurea*, *Meekiaria* sp. complex, and *T. deliciosa* (Tables S2 and S3).

Host-associated differentiation without geographic isolation is consistent with models of speciation by specialization in which adaptation to host chemistry or other plant traits has a stronger influence on divergence than geographic isolation (14–16). Mitochondrial host-associated  $\Phi_{ST}$  for New Guinea Lepidoptera, where significant (Table S2;  $x \pm SD = 0.223 \pm 0.113$ ,  $n = 15$ ), was somewhat lower than that for other sympatric host races, including gall midges ( $\Phi_{ST} = 0.789$ ) (17), apple maggot flies ( $\Phi_{ST} = 0.866$ ) (18), and golden rod gall makers ( $\Phi_{ST} = 0.504 \pm 0.370$ ,  $n = 3$ ) (10). Thus, it seems unlikely that the extent of host-associated differentiation is sufficient for recognition of numerous cryptic species that would alter estimates of host specificity for the New Guinea caterpillar community as whole. Even herbivores showing significant population genetic subdivision among hosts included oligophagous or polyphagous haplotypes (Fig. 3B), and most herbivore population genetic variance was distributed within rather than among host species (Table S2). This situation is very different from that for neotropical skipper butterfly, *Astraptes fulgerator*, in which multiple monophagous and cryptic species were recognized within a generalist herbivore (8). None of the New Guinea generalists (Table S1) could be decomposed into monophagous lineages, but, as in the case of goldenrod gall feeders (10), a substantial proportion of host clade specialists showed host-associated genetic differentiation.

**Isolation By Distance.** *Paraphomia disjuncta* (Fig. 3C and D) provides an example of geographic population genetic structure with no variation in host use across the species range. Cryptic lineages were distributed along an east-west axis, but all feed on *Maranga aleuritoides*. One lineage occurred almost exclusively at Elem, Niksek, Utai, Wamangu, and Wanang, whereas a second lineage was endemic to Yapsei, and a third lineage was endemic to Utai. *Adoxophyes thoracica*, *A. intacta* complex, *Mellea* sp. [THYR012], and *Phyliris helena* exhibited similar patterns, except that these species ranged in diet from oligophagy to polyphagy. Geographic population subdivision in the absence of host-associated differentiation appears to reflect limited gene flow and consequent genetic drift not explained by dietary specialization.

Species exhibiting genetic isolation by distance are ideal candidates for tests of phylogeographic hypotheses. The Sepik-Ramu river basin in northern New Guinea is a product of the gradual accretion of volcanic arc terranes to the Australian plate. The Adelbert, Bewani, and Torricelli mountain ranges (Fig. 1) each represent a series of formerly isolated oceanic islands that today form the northern boundary of the basin. These and geologic activity throughout the Miocene are considered factors promoting vicariant diversification (13), but intra-specific divergence in Lepidoptera appears to be more recent than these geologic events. Major tropical rivers have been proposed as Pleistocene barriers to gene flow in Amazonian butterflies (19, 20), but New Guinea Lepidoptera show no genetic subdivision of northern and southern populations across the 70-km-wide Sepik River floodplain (Fig. 1). Isolation by distance is instead structured along an east-west axis (Fig. 3C), which might be explained by the separation of eastern and western lowland forests during Holocene periods of elevated sea level when the Sepik-Ramu basin formed an extensive inland sea (21). Differences among Lepidoptera species in the extent of phylogeographic structure might be explained by relative dispersal ability.

**Localization and Specialization.** Some New Guinea Lepidoptera exhibit both geographic- and host-associated population genetic



of choice for detecting phylogeographic patterns (25) and we interpret the absence of mtDNA variation as evidence against a deep history of geographic or host-associated isolation of species populations. These assertions are amenable to testing with nuclear gene loci (26). Although more rapidly evolving markers, such as microsatellites or single nucleotide polymorphisms, could provide evidence of differentiation, we argue that this would be of relatively recent origin (27).

Evidence of long-distance dispersal in geographically widespread species is strengthened in cases where DNA sequences are available from elsewhere. For example, the most common *Glyphodes margaritaria* haplotype, occurring at all eight sites in New Guinea, was captured 1,500 km away in Queensland, Australia (GenBank no. GQ844281). The same *T. excelsalis* haplotype from northern New Guinea also was encountered at three sites in Australia, reaching a distance of 2,690 km from New Guinea (GenBank nos. GQ844282–GQ844288). A second haplotype, common to all eight northern New Guinea sites, was encountered at Misima Island (Fig. 1), a distance of 1,450 km from the westernmost site (GenBank no. GQ479200). A similar pattern was detected in *Talanga deliciosa*, where Misima haplotypes (Genbank no. GQ479199) were <0.01% divergent from mainland haplotypes. *Asota caricae* represents an extreme case against vicariance, with the same haplotype encountered in New Guinea also ranging from Taiwan to Australia (GenBank nos. GQ479199 and GQ844279), a distance of 5,340 km.

In the absence of dispersal, the extent of molecular divergence between New Guinean and Australian populations should date from that time when the northern lowlands were isolated from Australia by the uplift of the central New Guinea cordillera 4.7–5.8 mya (28, 29). Assuming a molecular clock and a rate of 1.5% divergence per million years for arthropod mtDNA (30), we predict 7.0%–8.7% divergence between New Guinean and Australian populations. Too few substitutions are observed between these populations to explain their history by geographic isolation. Recent long-distance dispersal in Lepidoptera provides a sharp contrast to vertebrates showing molecular divergence across the central cordillera that is consistent with vicariance (31, 32).

Low regional differentiation suggesting long-distance dispersal in Lepidoptera deviates from the prevailing view of biogeography as implied by extensive recognition of subspecies in the New Guinea fauna (33). On the other hand, patterns of sequence divergence in approximately one-third of sampled species are consistent with geographic population subdivision. Additional sampling of related taxa across the region, species distributed across the central cordillera, and local endemics is needed.

**Cryptic Species and  $\beta$ -Diversity.** Failure to recognize host-associated or geographically isolated cryptic species can result in underestimation of dietary specialization and endemism. The discovery of deeply diverged mtDNA lineages within species can motivate a morphological reassessment and the recognition of new species (34). Could this be the case for previous reports of low host specificity (5) and broad geographic distribution (1) that depart from prevailing views on endemism (13) and herbivore specialization in the tropics (6)? In an essentially random sample of widespread Lepidoptera species, most cryptic lineages within species were not local endemics, as might be expected by vicariance, but divergent and widespread haplotypes were often encountered in sympatry (Fig. 3 *G* and *H*). Such patterns could arise in relatively ancient taxa with secondary contact between previously isolated lineages resulting from rare, long-distance dispersal. We recognize the potential for species concepts to influence the detection of population genetic patterns, but we suspect that taxonomic reevaluation of New Guinea Lepidoptera will not substantially alter estimates of host specificity, because most mtDNA lineages within species are associated with broad and overlapping ranges of host species.

Determining whether contrary estimates of Lepidoptera host specificity from New Guinea (1) and the Americas (6) reflect major regional biogeographic differences requires evaluation based on comparable sampling efforts (35). A similar analysis of mtDNA sequences from the Guanacaste, Costa Rica caterpillar community is opportune (36). A comparison of Southeast Asian and Palearctic butterflies, for example, found remarkably little difference in host specificity between temperate and tropical faunas (37). Variation in diet and distribution of New Guinea herbivores agrees with that of goldenrod gallers in suggesting that not all speciation can be explained by specialization (14). Comparable DNA sequences from diverse taxa in ecological communities (8, 9) are the key to understanding whether species concepts or other factors, such as sampling methods or regional faunas (35), account for the divergent findings of recent studies.

**Synthesis.** High-throughput DNA sequencing and barcoding make the rapid assessment of large community samples containing diverse taxa feasible. Comparative population genetics of ecological guilds can reveal the generality (or lack thereof) in patterns of population differentiation that have bearing on major hypotheses for the origin and maintenance of species diversity (10). Identification of overall trends in communities requires unbiased and broader sampling than would typically be used for taxonomic purposes. Patterns of genetic variation sometimes fit predictions of ecological and phylogeographic theory, but the overlapping diets of numerous, broadly sympatric herbivores still requires explanation. Comparisons at the community level provide not the last step, but rather the first step in understanding processes responsible for diversification and specialization in an ecological context (14). Additional data from nuclear loci, large-scale comparative phylogeography using hierarchical Bayesian computation (38), and application of graph theory to test explicit hypotheses on the causes and consequences of genetic subdivision are logical next steps (39).

## Materials and Methods

**Caterpillar Sampling.** Sampling was conducted in the basins of the Sepik and Ramu rivers in Papua New Guinea (Fig. 1), across a 75,000-km<sup>2</sup> area of lowland terrain with continuous rainforest and wetland vegetation. The Sepik River is associated with a belt of swamps, lakes, and grasslands reaching 70 km in width that represents the only major discontinuity in the rainforest belt of the study area. Details are given in *SI Materials and Methods*. Large genera representing four plant families—*Ficus* (Moraceae), *Macaranga* (Euphorbiaceae), *Psychotria* (Rubiaceae), and *Syzygium* (Myrtaceae)—were sampled for folivorous, externally feeding larval Lepidoptera, hereinafter referred to as caterpillars. Caterpillars were hand-collected from ~1,500 m<sup>2</sup> of foliage per plant species. Each caterpillar was tested in a field laboratory for feeding on the plant species from which it was collected and reared to an adult whenever possible. Only caterpillars that fed were retained for study.

A total of 74,184 caterpillars and 370 morphospecies feeding on the target plant species were recorded, including 25,437 individuals and 346 morpho species reared to adults. Morphospecies as recognized in the field by parataxonomists were identified by dissection of genitalia, and whenever possible, by reference to type specimens or in consultation with experts. Adult legs were submitted to the University of Guelph for DNA sequencing according to published protocols (40). Twenty-eight taxa (Table S1) were collected in sufficient numbers for genetic analysis. New generic combinations include *Meekiaria purpurea*, "*Jodis*" (*s.l.*) *albifusa*, *Arctornis intacta* complex, and *Mellea nitida* (*Moth Taxonomy*).

**Population Genetic Analysis.** Analyses were based on a 575-bp alignment of mtDNA haplotypes following a test of species monophyly by Bayesian phylogenetic analysis (*SI Materials and Methods*). Median joining networks (41) were calculated to examine intraspecific variation in each of 28 species using default settings as implemented in Network 4.1 (Fluxus Technology). The geographic location and host association of each specimen carrying a given haplotype were coded to illustrate distribution and host range.

Analysis of molecular variance (AMOVA) was performed to assess how intraspecific genetic variation was partitioned among geographic localities and host plants (42). A geographically structured AMOVA partitioned genetic variation into components of within-site and among-site variation (two-level

AMOVA). Partitioning variance within and among hosts assessed the extent of host-associated differentiation. In some cases, it was possible to conduct a three-level AMOVA partitioning variance within host species, among host species, and among host clades (*SI Materials and Methods*). Sampling of Lepidoptera from particular host species at particular localities was insufficient to estimate covariance of geographic and host-associated population structure.

Pairwise *F* statistics were calculated among all sites and hosts where a particular species was recorded. In addition, Mantel tests of isolation by distance were performed to assess the significance of correlation among genetic distance, geographic distance, and host plant phylogenetic distance based on 100,000 permutations.

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# Supporting Information

Craft et al. 10.1073/pnas.0913084107

## SI Materials and Methods

**Study Area, Caterpillars, and Host Plants.** We outlined an approximately equidistant grid of eight sites with an average distance of 160 km between neighboring sites and with pairwise distances ranging from 59 to 513 km. Sites were located at <500 m above sea level in vegetation classified as mixed evergreen hill forest (1). The area has a human population of <10 people per km<sup>2</sup> and has <1 km of roads per 100 km<sup>2</sup>. The climate at these sites is humid, with a mean annual rainfall of 3,000–4,000 mm, a moderate dry season from July to September (monthly mean rainfall, <100 mm), and a mean monthly temperature of ~26 °C.

The study area is situated in a complex tectonic region at the convergence of two major plates, the Australian and Pacific, where ancient accretion of volcanic arc terranes to the central cordillera border the Sepik and Ramu river basins. Most of the study area was submerged from the Early Miocene until the Pliocene (2). Oceanic incursions across the northern lowlands during periods of elevated sea level continued until very recently, including a sea that stretched ~100 km inland and separated the Elem and Wamangu sites only 6,000 years ago (3). Climate and vegetation were very different during the last major glacial epoch than today, with a mosaic of open and closed-canopy forest covering the study area during a cooler and drier period about 17,000 years ago (4).

Caterpillars represent the most species-rich group of leaf-chewing insects in the study area (5). They were sampled from selected host plants during a single 3-month survey at each site between December 2001 and October 2005. Widespread and common caterpillar species, with numerous adults reared at each site, were the focus of intensive DNA barcoding, with approximately 10 individuals sequenced per site (Table S1). Herbivore diets ranged from monophagy to polyphagy (Table S1). Selected host plants are well represented in all stages of lowland rain forest succession (6) and together total at least 475 species in New Guinea (7). At each site, caterpillars were collected from 11 or 12 locally common plant species (4 or 5 *Ficus*, 3–5 *Macaranga*, 1 or 2 *Psychotria*, and 1 or 2 *Syzygium* species per site), except at Ohu and nearby Madang, where additional plant species were sampled as described previously (5). These species, composed of shrubs and small trees, represented <5% of the total basal area of the local woody vegetation (8). Geographic distributions of focal plant species ranged from widespread to narrowly endemic within the study area.

**Phylogenetic Analysis.** DNA barcodes were trimmed to 575 bp, the maximum contiguous sequence available for all taxa. A distribution of phylogenetic trees was obtained using MrBayes 3.1 (9, 10) to test monophyly of each species and to examine the extent of intraspecific variation. Modeltest 3.0 (11) was used to identify the most suitable model of nucleotide evolution. A model of molecular evolution, GTR + I + G, was selected on the basis of the Aikake information criterion and likelihood ratio tests. Two parallel analyses with six chains each were run for 5 × 10<sup>6</sup> generations. Trees were sampled every 1,000 generations. The first 4 × 10<sup>6</sup> generations were discarded as burn-in. The log-likelihood scores of sample points against generation time was plotted using Tracer 1.5 (<http://tree.bio.ed.ac.uk/software/tracer/>), to ensure that stationarity was achieved after the first 4 × 10<sup>6</sup> generations by checking whether the log-likelihood values of the sample points reached stable equilibrium. A majority-rule consensus tree with average branch lengths was calculated with the “sumt” option of MrBayes, and posterior probabilities were obtained for each clade from the remaining 2,000 trees.

Phylogenetic analysis of mitochondrial COI haplotypes confirmed the reciprocal monophyly of species and species complexes (Fig. S1), but caution should be exercised when evaluating phylogenetic relationships among species in community samples because of incomplete taxon sampling. Although the monophyly of Tortricidae and Thyrididae was recovered, a number of erroneous groupings were evident; for example, Geometridae were nested within Crambidae, and Noctuidae were polyphyletic. Moreover, Gelechiidae relationships were unresolved, and the monophyly of three families represented by only single species could not be assessed. Evolutionary interpretation of phylogenetic trends in dietary specialization and geographic distribution must be based not on Fig. S1, but rather on the best available estimate of phylogeny, which necessarily is a synthesis of independent studies and expert knowledge for particular groups.

Median joining networks for each species were drawn in Network Publisher (Fluxus Technology) and Adobe Illustrator CS4. We calculated K2P pairwise distances among haplotypes in PAUP\* (12). Divergence ≥2% is thought to reflect a degree of reproductive isolation in Lepidoptera (13, 14); however, because interspecific and intraspecific divergences of COI vary taxonomically (15, 16), we did not automatically conclude that lineages found to be ≥2% divergent are necessarily cryptic species without the presence of other corroborating evidence (e.g., morphology, diet). Pending thorough taxonomic reevaluation, we refer to cryptic lineages within species or species complexes as those separated by ≥2% divergence.

Population genetic analyses were performed using Arlequin version 3.11 (17) with the exception of haplotype number, which was determined using DnaSP version 4 (18). Nucleotide diversity (the average number of nucleotide differences among haplotypes) was calculated to estimate haplotype polymorphism within species.

Analysis of host-associated genetic differentiation involved the identification of host clades through molecular phylogenetic analyses of *ndhF* sequences for *Macaranga*, *rbcl* sequences for *Psychotria*, and ITS sequences for *Syzygium* and *Ficus*, as reported previously (19). Defining a host clade level in the Lepidoptera AMOVA is analogous to grouping sites within geographic regions; for example, in the case of the *Syzygium* specialist *Zeugma recusataria*, two host clades were defined in the AMOVA as *Syzygium malaccense* Merrill & Perry plus *Syzygium* sp. “SRB” and *Syzygium longipes* (Warb.) Merrill & Perry plus *Syzygium* cf. “stipulare.”

**Moth Taxonomy.** The taxonomic methods followed were as described by Miller et al. (20) and Holloway et al. (21), with initial sorting to morphospecies conducted by parataxonomists in the field. Species concepts following Miller (22) were based on male genitalia, and since 2003, on DNA barcodes as well. Comparisons of mitochondrial COI DNA sequence divergence with morphology were used to identify polymorphic species including cases of sexual dimorphism (13, 23). Lepidoptera species are illustrated at <http://www.entu.cas.cz/png/caterpillars>. Most species were identified with reference to type specimens in the Natural History Museum (London) and elsewhere. Pinned voucher specimens for DNA sequences are deposited in the Smithsonian Institution, Washington, DC, and additional voucher specimens of the same species are deposited in National Agriculture Research Institute, Port Moresby, Papua New Guinea. Notes on the a posteriori morphological evaluation of species complexes in light of mitochondrial evidence for cryptic diversity are included here to facilitate future taxonomic revision. Of the

four species complexes including cryptic taxa in need of description, *Arctornis* and *Meekiaria* were recognized in our earlier work, whereas *Jodis* and *Dichomeris* were discovered through the population genetic analysis described herein. We expect only a minimal impact of splitting these complexes on our previous ecological findings. Lepidoptera from Papua New Guinea, through Novotny et al. (8) were based on extensive use of genitalic morphology (22) and, since 2003, on DNA barcoding as well (23), to refine taxonomic species concepts.

**Choreutidae.** *Choreutis* cf. *anthorma* (Meyrick) is near but not *Choreutis anthorma*, as illustrated by Diakonoff (24) and Clarke (25).

**Crambidae.** “*Coelorhycidia*” *nitidalis* (Hampson) was matched to the type specimen, but the species does not belong in the genus *Coelorhycidia* and should be reassigned.

The *Meekiaria* species complex includes several species related to *M. purpurea* that have yet to be described. *M. purpurea* (Hampson) was recently matched to the type specimen by dissection of genitalia. This species was misplaced in the genus *Coelorhycidia* in earlier publications (19). This is a new generic combination with *Meekiaria* Munroe (26).

*Glyphodes margaritaria* (Clerck) is a widespread species reviewed by Sutrisno et al. (27).

*Talanga deliciosa* (Butler) is a widespread species placed in the genus *Agrioglypta* by Nielsen et al. (28), but because of problems in definition of *Agrioglypta* (27, 29), we retain it in *Talanga*.

*Talanga excelsalis* (Walker) is a widespread species (29). Placed in the genus *Agrioglypta* by Nielsen et al. (28), we retain it in *Talanga* due to problems with the definition of *Agrioglypta* (27, 29).

*Talanga sexpunctalis* is considered a widespread species (30) but appears to be a complex of species, including several in New Guinea. It remains unclear whether any of the New Guinea specimens are conspecific with *T. sexpunctalis* sensu stricto as described from the Andaman Islands. A specimen of *T. sexpunctalis* from Sulawesi (GenBank accession no. AB158250) also may represent a new species in the complex (27, 29).

**Gelechiidae.** *Dichomeris ochreoviridella* represents a species complex. The traditional concept of *D. ochreoviridella* (24, 30) is clearly two species according to male genitalia and mitochondrial DNA sequence divergence. The Australian *D. euchroa* Lower has sometimes been placed as a synonym (28) but appears to be a distinct species based on male genitalia. New Guinea specimens are placed in *Dichomeris* following Hodges (31).

*Dichomeris* sp. [XXXX068] and *Dichomeris* sp. [XXXX120] are undescribed species near *Dichomeris resignata* Meyrick, as illustrated by Clarke (25).

**Geometridae.** “*Jodis*” *albifusa*, new combination, is a complex of at least three species in New Guinea and at least three more in

Australia according to mitochondrial DNA sequence divergence. These species have been placed in *Thalassodes*, *Gelasma*, and *Maxates*, but genitalia point to a closer relationship with *Jodis* and *Berta* (32). One of the New Guinea species in particular has been confirmed as *albifusa* through comparison with the genitalia of the male type. After Holloway (32), we refer to the species complex as “*Jodis*” *albifusa* until the generic classification can be reviewed.

*Zeugma recusataria* Walker is a widespread species (33).

**Noctuidae.** *Asota carica* (Fabricius) and *A. plana* (Walker) are widespread species (34).

**Lycaenidae.** *Philiris helena* (Snellen) and *P. moira* (Grose-Smith) are species endemic to New Guinea (35).

**Lymantriidae.** *Arctornis intacta* complex is widespread in New Guinea and includes at least four morphospecies closely allied to, but not conspecific with, *A. intacta* (Walker) based on examination of the holotype at the University of Oxford Museum. This is a new generic combination in *Arctornis* (36).

**Pyralidae.** *Paraphomia disjuncta* Whalley appears to be a species endemic to New Guinea (37).

*Unadophanes trissomita* (Turner) is a widespread species identified by Horak based on her knowledge of the Australian fauna.

**Thyrididae.** *Addaea pusilla* (Butler) is a widespread species (28, 38).

*Mellea nitida* (Pagenstecher) is a new generic combination necessitated by the resurrection of *Mellea* (28). *M. ordinaria* (Warren) is considered a widespread species complex with a highly variable in wing pattern (39), but male genitalia do not show distinguishing characteristics among members of the complex. Thus, which of the New Guinea specimens are conspecific with the *ordinaria* type specimen is not clear, and the name is applied provisionally. *Mellea* sp. [THYR012] is a segregate of the *M. ordinaria* complex based on mitochondrial DNA sequence divergence.

*Rhodoneura aurata* (Butler) is a widespread species (28).

**Tortricidae.** *Adoxophyes thoracica* Diakonoff was identified based on male genitalia of the lectotype. Novotny et al. (40) identified the species as matching Diakonoff's illustration of female *A. nebrodes*, but we have confirmed that Diakonoff (41) misidentified the illustrated specimen.

*Adoxophyes* sp. nr. *orana* is evidently an undescribed species in the *A. orana* complex distinct from *A. orana* and *A. honmai* according to genitalia and mitochondrial DNA sequence divergence (42).

*Dudua* new species near *aprobola* (Meyrick) is quite similar to the widespread and polyphagous *D. aprobola* (30) but differs consistently by a clear white forewing mark.

*Ophiorrhabda deceptor* (Diakonoff) is a widespread species (43).

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**Table S1. New Guinea lowland rainforest Lepidoptera included in analyses of geographic and host-associated population genetic differentiation**

Species	Family	Individuals reared	Individuals sequenced	Haplotypes	Sites	Host species	Host range
<i>Addaea pusilla</i> (Butler)	Thyrididae	910	61	9	8	8	<i>Macaranga</i>
<i>Adoxophyes</i> sp. nr. <i>orana</i> (Fischer von Roeslerstamm) [TORT94, TORT066]	Tortricidae	75	36	6	5	8	Generalist
<i>Adoxophyes thoracica</i> Diakonoff	Tortricidae	37	37	9	6	12	Generalist
<i>Arctornis intacta</i> Walker complex	Lymantriidae	203	53	11	7	7	Generalist
<i>Asota carica</i> (F.)	Noctuidae	301	48	2	5	2	<i>Ficus</i>
<i>Asota plana</i> Walker	Noctuidae	150	40	1	5	2	<i>Ficus</i>
<i>Choreutis</i> sp. nr. <i>anthorma</i> (Meyrick) [TORT005]	Choreutidae	736	47	25	7	7	<i>Ficus</i>
" <i>Coelorrhycidia</i> " <i>nitidalis</i> Hampson	Crambidae	324	43	3	6	3	<i>Psychotria</i>
<i>Dichomeris ochreoviridella</i> (Pagenstecher) complex	Gelechiidae	1,069	60	22	8	8	<i>Macaranga</i>
<i>Dichomeris</i> sp. [XXXX068]	Gelechiidae	907	66	10	8	5	<i>Macaranga</i>
<i>Dichomeris</i> sp. [XXXX120]	Gelechiidae	77	37	8	6	9	<i>Macaranga</i>
<i>Dudua</i> sp. nov. nr. <i>aprobola</i> (Meyrick) [TORT143]	Tortricidae	160	30	7	5	2	<i>Syzygium</i>
<i>Glyphodes margaritaria</i> (Cramer)	Crambidae	225	50	2	8	7	<i>Ficus</i>
<i>Meekiaria purpurea</i> Hampson	Crambidae	1,178	41	12	7	4	<i>Psychotria</i>
<i>Meekiaria</i> sp. complex [CRAM041]	Crambidae	1,048	80	16	7	4	<i>Psychotria</i>
<i>Mellea nitida</i> (Pagenstecher)	Thyrididae	357	47	12	8	9	<i>Macaranga</i>
<i>Mellea ordinaria</i> (Warren)	Thyrididae	1,283	39	11	8	5	<i>Macaranga</i>
<i>Mellea</i> sp. [THYR012]	Thyrididae	101	35	12	5	2	<i>Macaranga</i>
<i>Ophiorrhabda deceptor</i> Diakonoff	Tortricidae	267	35	16	7	4	<i>Syzygium</i>
<i>Paraphomia disjuncta</i> Whalley	Noctuidae	394	39	19	6	1	<i>M. aleuritoides</i>
<i>Philiris helena</i> (Snellen)	Lycaenidae	189	41	20	6	6	<i>Macaranga</i>
<i>Philiris moira</i> Grose-Smith	Lycaenidae	85	29	5	4	6	<i>Ficus</i>
<i>Rhodoneura aurata</i> (Butler)	Thyrididae	484	47	19	7	3	<i>Macaranga</i>
<i>Talanga deliciosa</i> (Butler)	Crambidae	290	45	11	6	6	<i>Ficus</i>
<i>Talanga excelsalis</i> (Walker)	Crambidae	1,275	78	9	8	3	<i>Ficus</i>
<i>Talanga sexpunctalis</i> (Moore) complex	Crambidae	543	70	16	8	5	<i>Ficus</i>
" <i>Jodis</i> "(s.l.) <i>albifusa</i> (Warren) complex	Geometridae	247	41	14	6	3	Generalist
<i>Unadophanes trissomita</i> (Turner)	Pyralidae	482	37	15	6	6	<i>Macaranga</i>
<i>Zeugma recusataria</i> (Walker)	Geometridae	550	47	5	6	4	<i>Syzygium</i>

Species codes from Novotny et al. (8) are reported for new taxa and species complexes.

**Table S2. AMOVA for geographic and host-associated population genetic structure in New Guinea lowland rainforest Lepidoptera**

Species	Geography				Host association				
	% within sites	% among sites	$\Phi_{ST}$	% significant $F_{ST}$	% within species	% among species	% among clades	$\Phi_{ST}$	% significant $F_{ST}$
<i>Addaea pusilla</i>	96.95	3.05	0.031	3.6	56.22	36.12	7.66	0.438***	39.9
<i>Adoxophyes</i> sp. nr. <i>orana</i> [TORT94, TORT066]	77.75	22.25	0.222**	33.3	100.17	-0.17 <sup>†</sup>	NA	-0.02	0
<i>Adoxophyes thoracica</i>	58.26	41.74	0.417***	40	73.47	28.82	-2.29	0.265*	1.5
<i>Arctornis intacta</i> complex	73.05	26.95	0.269***	53.3	88.25	11.75	NA	0.117*	9.5
<i>Asota carica</i>	100.57	-0.57 <sup>†</sup>	-0.006	0	103.08	-3.08 <sup>†</sup>	NA	-0.031	0
<i>Choreutis</i> sp. nr. <i>anthorma</i> [TORT005]	96.59	3.41	0.034*	4.8	95.23	9	-4.23	0.048*	4.8
" <i>Coelorhycidia</i> " <i>nitidalis</i>	13.01	86.99	0.870***	60	59.56	40.44	NA	0.404***	33.3
<i>Dichomeris ochreoviridella</i> complex	77.55	22.45	0.224***	60.7	87.77	13.79	-1.56	0.122***	35.7
<i>Dichomeris</i> sp. [XXXX068]	43.66	56.34	0.563**	64.3	79.49	29.67	-9.16	0.205***	40
<i>Dichomeris</i> sp. [XXXX120]	100.96	-0.96 <sup>†</sup>	-0.010	0	106.6	-8.33	1.73	-0.066	0
<i>Dudua</i> sp. nov. nr. <i>aprobola</i> [TORT143]	68.72	31.28	0.313**	20	NA	NA	NA	NA	NA
<i>Glyphodes margaritaria</i>	94.51	5.49	0.055	0	112.07	-12.07 <sup>†</sup>	NA	-0.121	0
<i>Meekiaria purpurea</i>	71.57	28.43	0.284***	47.6	90.08	9.92	NA	0.099**	16.7
<i>Meekiaria</i> sp. complex [CRAM041]	97.57	2.43	0.024	0	63.46	6.19	30.35	0.365**	33.3
<i>Mellea nitida</i>	89.69	10.31	0.103*	14.3	87.95	12.05	NA	0.120*	8.3
<i>Mellea ordinaria</i>	75.86	24.14	0.241***	33.3	71.48	28.52	NA	0.285***	20
<i>Mellea</i> sp. [THYR012]	49.13	50.87	0.509***	90	NA	NA	NA	NA	NA
<i>Ophiorrhabda deceptor</i>	87.06	12.94	0.129**	20	95.72	4.28	NA	0.043	33.3
<i>Paraphomia disjuncta</i>	56.12	43.88	0.439***	60	NA	NA	NA	NA	NA
<i>Philiris helena</i>	87.89	12.11	0.121***	50	100.15	-0.15 <sup>†</sup>	NA	-0.002	0
<i>Philiris moira</i>	25.58	74.42	0.744***	66.7	81.18	18.82	NA	0.188	0
<i>Rhodoneura aurata</i>	61.89	38.11	0.381***	81	84.92	15.08	NA	0.151***	33.3
<i>Talanga deliciosa</i>	80.34	19.66	0.197***	70	69.39	25.09	5.52	0.306***	53.3
<i>Talanga excelsalis</i>	92.88	7.12	0.071	7.1	114.46	-14.46 <sup>†</sup>	NA	-0.145	0
<i>Talanga sexpunctalis</i> complex	98.45	1.55	0.016	10.7	99.38	0.62	NA	0.006	0
" <i>Jodis</i> " (s.l.) <i>albifusa</i> complex	68.85	31.15	0.311***	80	90.77	9.23	NA	0.092**	66.7
<i>Unadophanes trissomita</i>	85.59	14.41	0.144**	20	93.03	6.97	NA	0.070	6.7
<i>Zeugma recusataria</i>	105.1	-5.1 <sup>†</sup>	-0.051	0	70.28	29.72	NA	0.297**	50

NA, not applicable.

Significance: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

<sup>†</sup>Negative AMOVA covariance estimates may arise when components are approximately 0.

**Table S3. Qualitative descriptions of geographic and host-associated population genetic structure in New Guinea lowland rainforest Lepidoptera based on haplotype network analysis**

Species	Geography	Host association
<i>Addaea pusilla</i>	Two cryptic lineages, no structure	Two lineages with host-associated structure
<i>Adoxophyes</i> sp. nr. <i>orana</i> [TORT94, TORT066]	No structure	No structure
<i>Adoxophyes thoracica</i>	East-west structure	No structure
<i>Arctornis intacta</i> complex	Five cryptic lineages, some geographic structure	No structure
<i>Asota carica</i>	No structure	No structure
<i>Choreutis</i> sp. nr. <i>anthorma</i> [TORT005]	No structure, but extreme haplotype diversity	No structure
" <i>Coelorhycidia</i> " <i>nitidalis</i>	East-west structure, low diversity	Some host-associated structure
<i>Dichomeris ochreoviridella</i> complex	Two of five cryptic lineages with geographic structure	Four lineages with host-associated structure
<i>Dichomeris</i> sp. [XXXX068]	Two of three cryptic lineages with geographic structure	Two lineages with host-associated structure
<i>Dichomeris</i> sp. [XXXX120]	No structure	No structure
<i>Dudua</i> sp. nov. nr. <i>aprobola</i> [TORT143]	No structure	No structure
<i>Glyphodes margaritaria</i>	No structure	No structure
<i>Meekiaria purpurea</i>	Three cryptic lineages; east-west structure	One monophagous cryptic lineage
<i>Meekiaria</i> sp. complex [CRAM041]	Six cryptic lineages; endemic lineage in Utai	Two lineages with host-associated structure
<i>Mellea nitida</i>	No structure	No structure
<i>Mellea ordinaria</i>	Three cryptic lineages; east-west structure	Host-associated structure, but not partitioned among cryptic lineages
<i>Mellea</i> sp. [THYR012]	Private haplotypes	No structure
<i>Ophiorrhabda deceptor</i>	No structure	No structure
<i>Paraphomia disjuncta</i>	Three cryptic lineages, all haplotypes geographically structured	No structure
<i>Philiris helena</i>	Private haplotypes	No structure
<i>Philiris moira</i>	East-west structure	Some host-associated structure
<i>Rhodoneura aurata</i>	Four cryptic lineages; all haplotypes geographically structured	Three of four lineages with host-associated structure
<i>Talanga deliciosa</i>	Two cryptic lineages	Two lineages with host-associated structure
<i>Talanga excelsalis</i>	No structure	No structure
<i>Talanga sexpunctalis</i> complex	No structure	No structure
" <i>Jodis</i> " (s.l.) <i>albifusa</i> complex	Two cryptic lineages	No structure
<i>Unadophanes trissomita</i>	No structure	No structure
<i>Zeugma recusataria</i>	No structure	No structure

**Table S4. Mean pairwise nucleotide differences among individuals at eight sites for 28 lowland rainforest moth species**

Species	Ohu	Morox	Wanang	Yapsiei	Niksek	Elem	Utai	Wamangu
<i>Addaea pusilla</i>	4.68	7.17	0.40	5.78	6.33	0	6.50	6.17
<i>Adoxophyes</i> sp. nr. <i>orana</i>	0.81	0.76	NA	NA	NA	NA	0	0
<i>Adoxophyes thoracica</i>	0.79	0.44	NA	0	0	NA	0	NA
<i>Arctornis intacta</i> complex	0.44	5.51	NA	NA	13.28	10.80	4.06	16.20
<i>Asota carica</i>	0	NA	0	0	0.2	NA	NA	0
<i>Choreutis</i> sp. nr. <i>anthorma</i>	3.70	4.00	0	8.18	4.51	NA	3.40	2.00
" <i>Coelorhycidia</i> " <i>nitidalis</i>	0	0	0.39	0	NA	NA	0	0
<i>Dichomeris ochreoviridella</i> complex	13.78	21.00	0	20.94	15.94	14.13	0.91	16.78
<i>Dichomeris</i> sp. [XXXX068]	0.50	0	0.33	0.29	4.68	0.20	0	0.54
<i>Dichomeris</i> sp. [XXXX120]	0.68	0.50	NA	0	0.83	NA	0	0.67
<i>Dudua</i> sp. nov. nr. <i>aprobola</i>	0	1.00	NA	NA	0	NA	2.44	2.03
<i>Glyphodes margaritaria</i>	0	0	0.40	0	0	0	0	0
<i>Meekiaria purpurea</i>	13.17	0	NA	4.40	1.29	5.91	0.67	1.00
<i>Meekiaria</i> sp. complex	8.15	8.57	8.22	0.17	NA	0.50	19.16	14.28
<i>Mellea nitida</i>	5.14	NA	0	6.22	2.57	3.53	4.61	5.57
<i>Mellea ordinaria</i>	10.87	0.67	0	0.18	10.59	NA	8.20	NA
<i>Mellea</i> sp. [THYR012]	NA	0	NA	1.40	5.33	NA	0.44	5.43
<i>Ophiorrhabda deceptor</i>	0.33	2.57	NA	NA	3.43	3.00	3.16	0
<i>Paraphomia disjuncta</i>	NA	NA	1.25	0	2.07	0.40	6.80	4.67
<i>Philiris helena</i>	1.89	0	NA	11.19	NA	NA	4.46	4.29
<i>Philiris moira</i>	1.72	NA	NA	0	NA	NA	NA	0.20
<i>Rhodoneura aurata</i>	3.67	0.22	NA	0.68	7.17	0	4.14	8.20
<i>Talanga deliciosa</i>	0.25	NA	0.25	NA	4.26	NA	1.89	6.11
<i>Talanga excelsalis</i>	0.47	0.78	1.17	1.56	0	0.56	0.87	0.89
<i>Talanga sexpunctalis</i> complex	1.22	1.80	1.82	1.53	1.89	1.71	1.64	0.95
" <i>Jodis</i> " (s.l.) <i>albifusa</i> complex	9.86	NA	7.05	NA	NA	1.00	9.11	0.29
<i>Unadophanes trissomita</i>	NA	NA	NA	3.93	4.67	1.18	3.00	0.73
<i>Zeugma recusataria</i>	0.47	0.20	0	NA	NA	0	0.44	0

NA, not applicable.

**Table S5. Nucleotide diversity per site for 28 lowland rainforest moth species and species complexes**

Species	Ohu	Morox	Wanang	Yapsiei	Niksek	Elem	Utai	Wamangu
<i>Addaea pusilla</i>	0.011	0.017	0.001	0.014	0.015	0	0.015	0.015
<i>Adoxophyes</i> sp. nr. <i>orana</i>	0.001	0.001	NA	NA	NA	NA	0	0
<i>Adoxophyes thoracica</i>	0.001	0.0008	NA	0	0	NA	0	NA
<i>Arctornis intacta</i> complex	0.0008	0.010	NA	NA	0.024	0.020	0.007	0.030
<i>Asota carica</i>	0	NA	0	0	0.0004	NA	NA	0
<i>Choreutis</i> sp. nr. <i>anthorma</i>	0.007	0.008	0	0.016	0.009	NA	0.007	0.004
" <i>Coelorhycidia</i> " <i>nitidalis</i>	0	0	0.0007	0	NA	NA	0	0
<i>Dichomeris ochreoviridella</i> complex	0.026	0.040	0	0.039	0.030	0.027	0.002	0.032
<i>Dichomeris</i> sp. [XXXX068]	0.001	0	0.0006	0.0005	0.009	0.0004	0	0.001
<i>Dichomeris</i> sp. [XXXX120]	0.001	0.0009	NA	0	0.001	NA	0	0.001
<i>Dudua</i> sp. nov. nr. <i>aprobola</i>	0	0.002	NA	NA	0	NA	0.004	0.004
<i>Glyphodes margaritaria</i>	0	0	0.0007	0	0	0	0	0
<i>Meekiaria purpurea</i>	0.027	0	NA	0.009	0.003	0.012	0.001	0.002
<i>Meekiaria</i> sp. complex	0.1087	0.1143	0.1096	0.0022	NA	0.0067	0.2555	0.1905
<i>Mellea nitida</i>	0.010	NA	0	0.012	0.005	0.007	0.009	0.010
<i>Mellea ordinaria</i>	0.022	0.001	0	0.0004	0.022	NA	0.017	NA
<i>Mellea</i> sp. [THYR012]	NA	0	NA	0.003	0.010	NA	0.0008	0.010
<i>Ophiorrhabda deceptor</i>	0.0006	0.005	NA	NA	0.007	0.006	0.006	0
<i>Paraphomia disjuncta</i>	NA	NA	0.002	0	0.004	0.0007	0.013	0.009
<i>Philiris helena</i>	0.003	0	NA	0.020	NA	NA	0.008	0.008
<i>Philiris moira</i>	0.003	NA	NA	0	NA	NA	NA	0.0004
<i>Rhodoneura aurata</i>	0.007	0.0004	NA	0.001	0.014	0	0.008	0.016
<i>Talanga deliciosa</i>	0.0005	NA	0.0005	NA	0.009	NA	0.004	0.013
<i>Talanga excelsalis</i>	0.0009	0.002	0.002	0.003	0	0.001	0.002	0.002
<i>Talanga sexpunctalis</i> complex	0.002	0.004	0.004	0.003	0.004	0.003	0.003	0.002
" <i>Jodis</i> " (s.l.) <i>albifusa</i> complex	0.022	NA	0.015	NA	NA	0.002	0.020	0.0006
<i>Unadophanes trissomita</i>	NA	NA	NA	0.007	0.009	0.002	0.006	0.001
<i>Zeugma recusataria</i>	0.001	0.0005	0	NA	NA	0	0.001	0

NA, not applicable.

**Table S6. Numbers of Lepidoptera mtDNA haplotypes (and individuals) sampled from eight lowland rainforests in New Guinea**

Species	Ohu	Morox	Wanang	Yapsiei	Niksek	Elem	Utai	Wamangu
<i>Addaea pusilla</i>	4 (14)	3 (4)	2 (5)	3 (9)	3 (9)	NA	3 (8)	4 (9)
<i>Adoxophyes cf orana</i>	4 (19)	3 (10)	NA	NA	NA	NA	1 (1)	1 (6)
<i>Adoxophyes thoracica</i>	6 (20)	3 (9)	NA	1 (1)	1 (4)	NA	1 (3)	NA
<i>Arctornis intacta</i> complex	3 (9)	3 (10)	NA	NA	4 (10)	2 (5)	5 (9)	3 (5)
<i>Asota carica</i>	1 (10)	NA	1 (9)	1 (10)	2 (10)	NA	NA	1 (9)
<i>Asota plana</i>	1 (10)	NA	1 (9)	1 (10)	1 (2)	NA	NA	1 (10)
<i>Choreutis</i> sp. nr. <i>Anthorma</i>	8 (12)	6 (6)	1 (1)	7 (10)	8 (10)	NA	4 (5)	2 (3)
" <i>Coelorhycidia</i> " <i>nitidalis</i>	1 (9)	1 (3)	2 (9)	1 (8)	NA	NA	1 (5)	1 (9)
<i>Meekiaria purpurea</i>	3 (4)	1 (2)	NA	2 (5)	3 (10)	5 (10)	3 (6)	2 (4)
<i>Meekiaria</i> sp. Complex	6 (20)	2 (7)	3 (11)	2 (12)	NA	2 (4)	6 (11)	2 (15)
<i>Dichomeris ochreoviridella</i> complex	3 (9)	2 (2)	1 (2)	7 (9)	4 (9)	5 (10)	3 (10)	7 (9)
<i>Dichomeris</i> sp. [XXXX068]	2 (9)	1 (8)	2 (6)	2 (7)	3 (8)	2 (10)	1 (10)	2 (8)
<i>Dichomeris</i> sp. [XXXX120]	3 (8)	2 (4)	NA	1 (1)	4 (9)	NA	1 (9)	3 (6)
<i>Dudua</i> sp. nov. nr. <i>Aprobola</i>	1 (9)	2 (2)	NA	NA	1 (1)	NA	5 (9)	4 (9)
<i>Glyphodes margaritaria</i>	1 (9)	1 (1)	2 (5)	1 (10)	1 (3)	1 (5)	1 (7)	1 (10)
<i>Mellea nitida</i>	2 (7)	NA	1 (1)	4 (9)	2 (7)	3 (6)	4 (9)	6 (8)
<i>Mellea ordinaria</i>	5 (6)	2 (3)	1 (2)	2 (11)	4 (12)	NA	3 (5)	NA
<i>Mellea</i> sp.	NA	1 (5)	NA	3 (10)	2 (4)	NA	3 (9)	5 (7)
<i>Ophiorrhabda deceptor</i>	2 (6)	6 (7)	NA	NA	6 (8)	2 (2)	6 (10)	1 (2)
<i>Paraphomia disjuncta</i>	NA	NA	4 (8)	1 (10)	5 (8)	2 (5)	5 (5)	3 (3)
<i>Philiris helena</i>	6 (10)	1 (1)	NA	5 (7)	NA	NA	10 (18)	3 (7)
<i>Philiris moira</i>	3 (9)	NA	NA	1 (10)	NA	NA	NA	2 (10)
<i>Rhodoneura aurata</i>	3 (4)	2 (9)	NA	3 (8)	6 (9)	1 (4)	5 (8)	3 (5)
<i>Talanga deliciosa</i>	2 (8)	NA	2 (8)	NA	3 (10)	NA	7 (9)	2 (10)
<i>Talanga excelsalis</i>	3 (12)	3 (9)	4 (9)	4 (10)	1 (9)	3 (10)	3 (10)	4 (9)
<i>Talanga sexpunctalis</i> complex	5 (10)	4 (5)	4 (10)	7 (10)	6 (9)	6 (8)	6 (10)	3 (7)
" <i>Jodis</i> " (s.l.) <i>albifusa</i> complex	4 (8)	NA	5 (7)	NA	NA	2 (9)	5 (10)	2 (7)
<i>Unadophanes trissomita</i>	NA	NA	NA	4 (6)	3 (4)	4 (8)	5 (9)	3 (10)
<i>Zeugma recusataria</i>	3 (12)	2 (10)	1 (1)	NA	NA	1 (6)	3 (9)	1 (7)

NA, not applicable.