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

Kathleen J. Craft[a], Steffen U. Pauls[b], Karolyn Darrow[c], Scott E. Miller[c], Paul D. N. Hebert[d], Lauren E. Helgen[c], Vojtech Novotny[e], and George D. Weiblen[a,1]

Departments of [a]Plant Biology and [b]Entomology, University of Minnesota, Saint Paul, MN 55108; [c]National Museum of Natural History, Smithsonian Institution, Washington, DC 20013; [d]Biodiversity Institute of Ontario, University of Guelph, Guelph, ON N1G 2W1, Canada; and [e]Institute of Entomology, Czech Academy of Sciences and Department of Zoology, University of South Bohemia, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic

Communicated by Hans R. Herren, Millennium Institute, Arlington, VA, January 5, 2010 (received for review November 23, 2008)

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)

Author contributions: S.E.M., V.N., and G.D.W. designed research; K.J.C., S.U.P., K.D., S.E.M., V.N., and G.D.W. performed research; K.D., S.E.M., P.D.N.H., and L.E.H. contributed new reagents/analytic tools; K.J.C., S.U.P., and G.D.W. analyzed data; and K.J.C., S.U.P., and G.D.W. wrote the paper.

The authors declare no conflict of interest.

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).

[1]To whom correspondence should be addressed. E-mail: gweiblen@umn.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0913084107/DCSupplemental.

distance ≥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 Φ_{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 ≥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, "*Coelorhycidia*" *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 Φ_{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

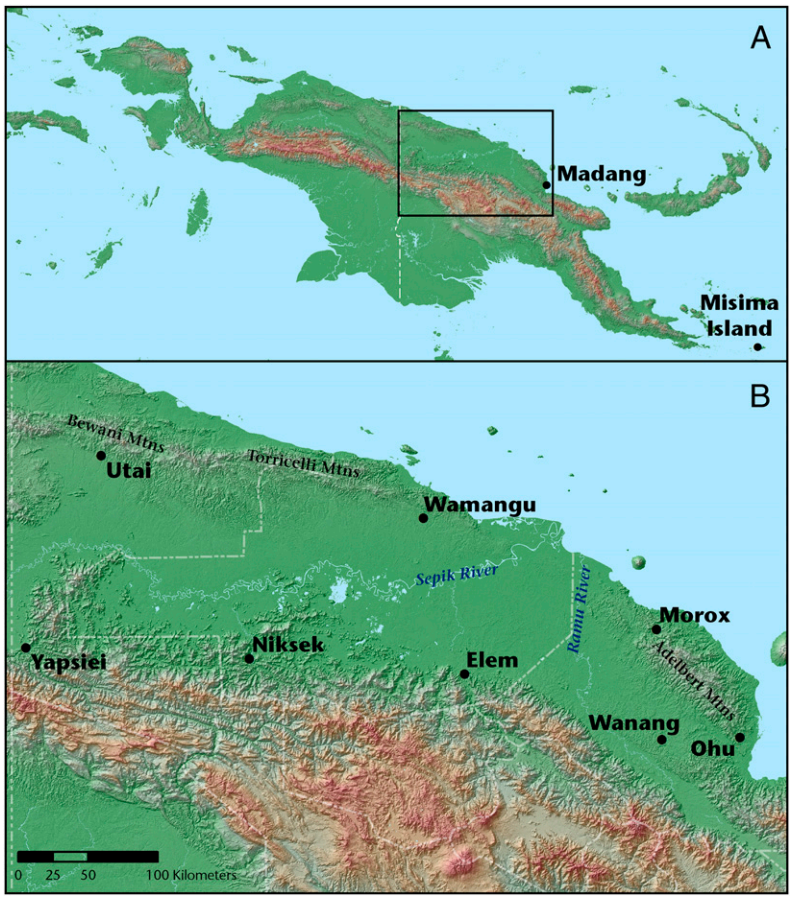

Fig. 1. (*A*) The island of New Guinea, with a rectangle marking the study area in the northern lowlands near the coastal town of Madang, Papua New Guinea. (*B*) Eight sites located in the Sepik-Ramu river basin that were the focus of Lepidoptera sampling by Novotny et al. (7).

structure among host clades; for example, 30.35% of the genetic variance in *Meekiaria* sp. complex was distributed among *Psychotria* host clades. Host-associated Φ_{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, *Arctornis 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. 3 *A* and *B*), in which two broadly sympatric lineages exhibited host-associated genetic differentiation. Although the lineages shared some host species in common, there were also exclusive associations with different hosts in sympatry. At Ohu, 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).

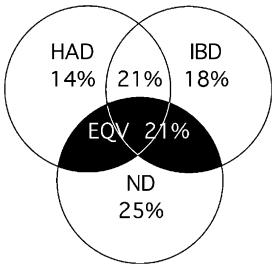

Fig. 2. Percentage of 28 Lepidoptera species exhibiting host-associated differentiation (HAD), isolation by distance (IBD), no differentiation (ND), and equivocal population genetic patterns (EQV) where analyses of molecular variance conflicted with haplotype networks.

Host-associated 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 Φ_{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. 3*B*), 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. 3 *C* 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 *Philiris 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 intraspecific 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. 3*C*), 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

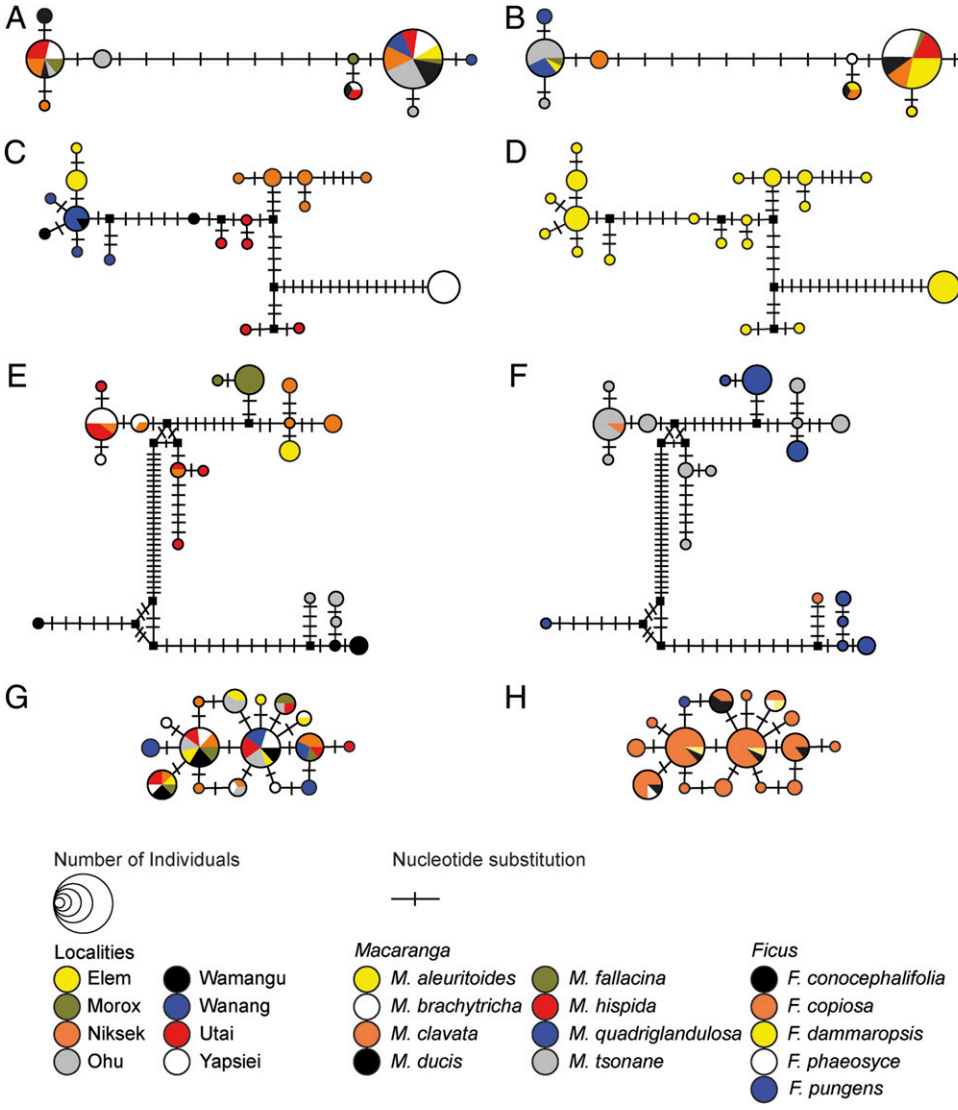

Fig. 3. mtDNA haplotype networks of four selected species: *A. pusilla* (*A* and *B*), *P. disjuncta* (*C* and *D*), *R. aurata* (*E* and *F*), and *T. sexpunctalis* (*G* and *H*). Colored circles represent haplotypes. Colors represent the geographic origin of specimens (*Left*) and host association of specimens (*Right*). Diameter is relative to the number of specimens carrying a particular haplotype where the smallest circle represents a single individual and the largest 35 individuals. Lines between haplotypes represent genetic distances between haplotypes; each mark represents a single nucleotide substitution. Black squares represent median vectors.

structures, as illustrated by *Rhodoneura aurata* (Fig. 3 *E* and *F*). This species consists of several geographically isolated populations characterized by private haplotypes. Niksek, Yapsiei, and Utai haplotypes were found primarily on *M. tsonane*, whereas Morox, Elem, and Wamangu haplotypes were found exclusively on *M. quadriglandulosa*. In this case, dietary specialization is explained by turnover in preferred host species across the geographic range of the herbivore. *M. quadriglandulosa* occurs only in the eastern localities and is replaced by *M. tsonane* in the west. Feeding records indicate that these locally endemic hosts are preferred to the widespread *M. clavata*. Several other species, including "*Coleorhycidia*" *nitidalis*, *Dichomeris ochreoviridella* complex, *Dichomeris* sp. [XXXX068], *Mellea ordinaria*, and *Philiris moira*, exhibit similar geographic variation in host preferences.

Such patterns of genetic subdivision can be explained by dispersal limitation combined with the evolution of dietary specialization, as predicted by geographic models of coevolution (22). Geographic variation in host preferences of specialists is more likely to evolve when populations are geographically isolated and host plants are beta-diverse. The complex tectonic history of New Guinea is considered an engine for allopatric speciation (13) that could accelerate dietary specialization in the presence of geographic turnover in the host plant community. Dietary specialization is a strong correlate of diversification (23, 24). Ecological theory further predicts that greater interspecific resource competition in diverse tropical communities compared with relatively depauperate temperate communities drives the evolution of greater dietary specialization in the tropics (3, 4). A substantial component of New Guinea Lepidoptera is neither specialized nor isolated by distance, however.

Dietary Generalization and Dispersal. The pattern of neither geographic nor host-associated population subdivision is illustrated by *Talanga sexpunctalis* (Fig. 3 *G* and *H*). The species was reared from six *Ficus* species and all eight sites, with individual haplotypes occurring on up to three host species and at up to seven sites. Geographically widespread haplotypes feeding on multiple host species also were observed in *Asota carica, A. plana, Choreutis* sp. nr. *anthorma, Dichomeris* sp. [XXXX120], *Glyphodes margaritaria,* and *T. excelsalis*. The observation of the same haplotype on multiple host species and/or at multiple sites provides strong evidence against dietary specialization and/or geographic isolation (Fig. 3 *A*, *D*, *G*, and *H*). Haplotypes shared among host species provide molecular genetic corroboration of dietary breadth (9), whereas haplotypes shared among localities indicate recent dispersal.

Such inferences from mtDNA are not likely to be challenged by additional nuclear DNA sequences, because longer coalescence times make nuclear markers lagging indicators of population differentiation. Mitochondrial DNA remains the marker

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 β-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 form 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^2 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^2 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.

ACKNOWLEDGMENTS. We thank the New Guinea Binatang Research Center staff, the more than 150 insect collectors from communities named in Fig.1, J. D. Holloway, M. Horak, J. Miller, J. Brown, E.G. Munroe, M. Shaffer, and K. Tuck for technical and taxonomic assistance. We also thank three anonymous reviewers, V. Hypsa, and P. Tiffin for their comments on the manuscript. This paper is based on work supported by the National Science Foundation under Grants DEB 9628840, 9707928, 0211591, and 0515678. The work was also supported by grants from the Grant Agencies of the Czech Republic (206/09/0115 and 206/08/H044), Czech Academy of Sciences (AA600960712 and AV0Z50070508), Czech Ministry of Education (LC06073, ME916, ME9082, and MSM6007665801), Darwin Initiative for the Survival of Species, the David and Lucile Packard Fellowship in Science and Engineering (to G.D.W.), the Gordon and Betty Moore Foundation (to P.D.N.H.), and the German Academy of Sciences Leopoldina (BMBF-LPD 9901/8-169, to S.U.P.). Finally, we acknowledge the cooperation of the Papua New Guinea Department of Environment and Conservation, National Research Institute, Forest Research Institute, and National Agricultural Research Institute.

1. Novotny V, et al. (2007) Low beta diversity of herbivorous insects in tropical forests. *Nature* 448:692–695.
2. Tilman D (1994) Competition and biodiversity in spatially structured habitats. *Ecology* 75:2–16.
3. MacArthur RH, Levins R (1967) The limiting similarity, convergence, and divergence of coexisting species. *Am Nat* 101:377–385.
4. Connell JH (1978) Diversity in tropical rain forests and coral reefs. *Science* 199: 1302–1310.
5. Novotny V, et al. (2006) Why are there so many species of herbivorous insects in tropical rainforests? *Science* 313:1115–1118.
6. Dyer LA, et al. (2007) Host specificity of Lepidoptera in tropical and temperate forests. *Nature* 448:696–700.
7. Novotny V, et al. (2002) Low host specificity of herbivorous insects in a tropical forest. *Nature* 416:841–844.
8. Hebert PDN, et al. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper buterfly *Astraptes fulgerator*. *Proc Natl Acad Sci USA* 101: 14812–14817.
9. Hulcr J, et al. (2007) DNA barcoding confirms polyphagy in a generalist moth, *Homona mermerodes* (Lepidoptera: Tortricidae). *Mol Ecol Notes* 7:549–557.
10. Stireman JO, Nason JD, Heard SB (2005) Host-associated genetic differentiation in phytophagous insects: General phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. *Evolution* 59:2573–2587.
11. Nason JD, Hamrick JL, Fleming TH (2002) Historical vicariance and postglacial colonization effects on the evolution of genetic structure in *Lophocerus*, a Sonoran Desert columnar cactus. *Evolution* 56:2214–2226.
12. Gressitt JL (1982) *Biogeography and Ecology of New Guinea* (W. Junk Publishers, The Hague).
13. Heads M (2006) Biogeography, ecology and tectonics in New Guinea. *J Biogeogr* 33: 957–958.
14. Tilmon KJ (2007) *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects* (University of California Press, Berkeley, CA).
15. Becerra JX, Venable DL (1999) Macroevolution of insect–plant associations: The relevance of host biogeography to host affiliation. *Proc Natl Acad Sci USA* 96: 12626–12631.
16. Coyne JA, Orr HA (2004) *Speciation* (Sinauer Associates, Sunderland, MA).
17. Dorchin N, et al. (2009) Behavioural, ecological and genetic evidence confirm the occurrence of host-associated differentiation in goldenrod gall-midges. *J Evol Biol* 22: 729–739.
18. Xianfa X, et al. (2009) Hawthorn-infesting populations of *Rhagoletis pomonella* in Mexico and speciation mode plurality. *Evolution* 61:1091–1105.
19. Gascon C, et al. (2000) Riverine barriers and the geographic distribution of Amazonian species. *Proc Natl Acad Sci USA* 97:13672–13677.
20. Hall JPW, Harvey DJ (2002) The phylogeography of Amazonia revisited: New evidence from riodinid butterflies. *Evolution* 56:1489–1497.
21. Dickinson WR (2001) Paleoshoreline record of relative Holocene sea levels on Pacific islands. *Earth Sci Rev* 55:191–234.
22. Thompson JN (2005) *The Geographic Mosaic of Coevolution* (University of Chicago Press, Chicago).
23. Ehrlich P, Raven P (1964) Butterflies and plants: A study in coevolution. *Evolution* 18: 586–608.
24. Janz N, Nylin S, Wahlberg N (2006) Diversity begets diversity: Host expansions and the diversification of plant-feeding insects. *BMC Evol Biol* 6:1–10.
25. Zink RM, Barrowclough GF (2008) Mitochondrial DNA under siege in avian phylogeography. *Mol Ecol* 17:2107–2121.
26. Brito P, Edwards SV (2008) Multilocus phylogeography and phylogenetics using sequence-based markers. *Genetica* 135:439–455.
27. Feder JL, et al. (2003) Allopatric genetic origins for sympatric host-plant shifts and race formation in *Rhagoletis*. *Proc Natl Acad Sci USA* 100:10314–10319.
28. Haig DW, Medd D (1996) Latest miocene to early pliocene bathymetric cycles related to tectonism, Puri anticline, Papuan basin, Papua New Guinea. *Aust J Earth Sci* 43: 451–465.
29. Hill KC, Gleadow AJW (1989) Uplift and thermal history of the Papuan Fold Belt, Papua New Guinea: Apatite fission track analysis. *Aust J Earth Sci* 36:515–539.
30. Quek SP, Davies SJ, Itino T, Pierce NE (2004) Codiversification in an ant–plant mutualism: Stem texture and the evolution of host use in *Crematogaster* (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). *Evolution* 58:554–570.
31. Rawlings LH, Donnellan SC (2003) Phylogeographic analysis of the green python, *Morelia viridis*, reveals cryptic diversity. *Mol Phylogenet Evol* 27:36–44.
32. Dumbacher JP, Fleischer RC (2001) Phylogenetic evidence for colour pattern convergence in toxic pitohuis: Mullerian mimicry in birds? *Proc R Soc Lond B Biol Sci* 268:1971–1976.
33. Parsons MJ (1998) *The Butterflies of Papua New Guinea: Their Systematics and Biology* (Academic, San Diego).
34. Hajibabaei M, et al. (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proc Natl Acad Sci USA* 103:968–971.
35. Corlett RT, Primack RB (2006) Tropical rainforests and the need for cross-continental comparisons. *Trends Ecol Evol* 21:104–110.
36. Janzen DH, et al. (2009) Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. *Mol Ecol Resources* 9:1–26.
37. Fiedler K (1998) Diet breadth and host plant diversity of tropical- vs. temperate-zone herbivores: South-East Asian and West Palaearctic butterfiles as a case study. *Ecol Entomol* 23:285–297.
38. Hickerson MJ, Dolman G, Moritz C (2006) Comparative phylogeographic summary statistics for testing simultaneous vicariance. *Mol Ecol* 15:209–223.
39. Dyer RJ, Nason JD (2004) Population graphs: The graph theoretic shape of genetic structure. *Mol Ecol* 13:1713–1727.
40. Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Mol Ecol Notes* 7:355–364.
41. Bandelt H, Forster P, Rohl A (1999) Median-joining networks for intraspecific phylogenies. *Mol Biol Evol* 16:37–48.
42. Excoffier L, Laval G, Schneider S (2005) Arlequin version 3.0: An integrated software package for population genetics data analysis. *Evol Bioinform Online* 1:47–50.