1 Limited erosion of genetic and species diversity from small forest patches: sacred forest
2 groves in an Afrotropical biodiversity hotspot have high conservation value for butterflies.

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Abstract

Sacred forest groves are often located in some of the world's hottest hotspots of biodiversity, and consequently have high potential conservation value. Recent efforts to quantify their value have focused nearly exclusively on a single component of diversity, species diversity within communities, which may or may not be an effective proxy for a second fundamental component of diversity, genetic diversity within populations. We studied fruit-feeding butterfly communities to simultaneously assess to what extent five small sacred groves have retained the level of species and genetic diversity found in two much larger forest reserves. We additionally evaluate whether measures correlate across habitat fragments to investigate how closely these two components of diversity mirror each other. We quantified the diversity and composition of the fruit-feeding butterfly communities at each site and also the haplotype diversity within three specific species that differ with respect to their sensitivity to habitat fragmentation. Of the multiple measures of species and genetic diversity computed, only rarefied species richness was correlated with forest fragment size and even in this case the relationship was weak. Importantly, the limited decline in species richness documented in the sacred groves was not due to species replacements, whereby common, broadly distributed, generalists supplanted more vulnerable species in these communities. Although similar processes are known to drive declines in both species and gene diversity, we found only limited evidence of positive species-genetic diversity correlations (SGDCs), and only in the species most sensitive to fragmentation. Thus, a conservation strategy that emphasizes species complementarity or richness may be ineffective at capturing other critical levels of biodiversity. Overall, our findings demonstrate that even very small forest patches can have a conservation value that rivals that of much larger forest reserves. The implementation of official national and international initiatives that preserve and strengthen existing community-based conservation practices are critically needed to ensure that indigenous conservation areas persist into the future.

Key Words: Sacred groves, diversity-area relationships, species-genetic diversity correlations, forest fragmentation, community conservation, Afrotropics

1. Introduction

Sacred natural areas set aside and protected by indigenous cultures represent the earliest forms of environmental conservation in the world (Posey, 1999). Sanctions and taboos, which in many cases have been enforced over centuries, restricted entry to these sites except for a few specific individuals or limited groups, and only for specific events. Most sacred sites have their origins in religious or traditional belief systems, key historical events, or are royal burial grounds, and conservation *per se* was rarely the intended objective (Bharuch, 1999; Ntiama-Baidu, 2001; Dudley et al., 2009). Nonetheless, the traditional laws strictly enforced by local communities fostered the long term persistence of these natural areas and coincidentally also secured their role as repositories of local biodiversity and as *de facto* protected areas.

Although indigenous protected areas take on a wide variety of forms (Mgumia and Oba, 2003; Shen et al., 2010), sacred forest groves are a common type found widely throughout the forest zones of Africa and Asia (Lebbie and Freudenberger, 1996; Hughes and Chandran, 1998; Malhotra et al., 2001; Mgumia and Oba, 2003; Bhagwat and Rutte, 2006; Hu et al., 2011). Sacred groves are small areas of forest habitat that are allied with and hold special spiritual significance for local village communities. Their general abundance links to the historical practice of communities in close proximity of forests to hold initiation rituals and secret ceremonies within the boundaries of the forest, and to section off specific areas as burial grounds for chiefs and other village elites. Most sacred groves currently exist as isolated forest remnants embedded within a transformed, non forest landscape matrix. In some regions, these relict patches constitute the only remaining

examples of what were formerly much larger, or even extensive, expanses of old growth forest (UNESCO, 2003; Cardelús et al., 2013).

Interest in the potential conservation value of sacred forest groves has increased markedly over the past decade (UNESCO, 2003; Bhagwat and Rutte, 2006; Verschuuren, 2010). This heightened conservation attention seems well deserved given their long history of protection, their tight linkage with local communities and locally-administered management, and their prevalence in some of the world's hottest hotspots of biodiversity. But most sacred groves are very small (some are only a few hectares) and, consequently, expected to harbor reduced species and genetic diversity relative to larger tracts of forest habitat, owing to the multiple and interacting ecological, demographic, and evolutionary processes that promote loss of species and genetic variation from small, isolated habitat fragments (MacArthur and Wilson, 1967; Avise, 1994; Frankham, 1995; Rosenzweig, 1995; Turner, 1996; May and Stumpf, 2000). As such, their value may only be apparent when the biodiversity of multiple groves is considered collectively, when they happen to harbor relict populations of rare or declining species, or when they facilitate gene flow and connectivity of fragmented forest populations across the broader landscape.

Determining the importance of sacred groves for forest conservation has taken on an increased urgency. The traditional beliefs that have long protected these unique, community-based reserves have decayed in concert with the influx of western influences and immigration into local communities by those holding different value systems (Ntiama-Baidu, 2001; Dudley et al., 2010). Consequently, many groves are rapidly being degraded and/or lost. Despite their obvious and irreplaceable cultural significance, loss of the world's sacred groves may have limited consequences for conservation if, as predicted, the biodiversity they harbor is generally found to be much reduced to that in large expanses of forest or if their resident communities are largely dominated by widespread, common generalists. Although studies of sacred groves to date have tended to support this expectation of reduced diversity, these have been limited in number and in their geographic and taxonomic scope (e.g. UNESCO, 2003; Bhagwat and Rutte, 2006; Dudley et al., 2009; Shahabuddin and Rao, 2010) and may not reflect their conservation value more generally. Previous studies have also focused nearly entirely on species diversity, and often on only one measure, species richness, but species diversity is just one important element of biodiversity and may or may not adequately reflect other key levels of diversity.

Here, we quantify the extent to which small, individual forest groves retain the levels of butterfly biodiversity found in much larger forest reserves. To our knowledge, this study is the first that simultaneously considers both genetic diversity within species and species diversity within communities, the two most fundamental levels of biodiversity, to assess the value of indigenous conservation areas. We focus on a relatively understudied region of the world where a large number of sacred forest groves are known to occur, Ghana, West Africa. Diversity associated with five sacred groves was compared to that of two much larger governmental forest reserves. Species diversity was assessed by sampling the species-rich community of fruit-feeding forest butterflies associated with each forest fragment. The species diversity component herein extends Bossart et al. (2006), which was a more descriptive, less thorough characterization of species diversity in four sacred groves. Genetic diversity was assessed by characterizing the population genetic profiles of three co-distributed forest butterfly species that differ with respect to their relative sensitivity to habitat fragmentation. A second goal of the study was to investigate whether levels of species and gene diversity, which are influenced by similar processes (Vellend et al., 2014), were correlated within and between forest fragments. High correlation would imply that conservation evaluation could be streamlined as one measure of diversity could serve as an effective proxy for assessing the general conservation value of habitat fragments.

2. Materials Methods

2.1. Study sites and sampling

Ghana lies at the eastern most extent of the Upper Guinean forests of western West Africa, a region that has been experiencing one of the highest rates of deforestation in the world (Myers et al., 2000; Poorter et al., 2004; FAO, 2009). Although estimates vary, by all accounts the large majority of Ghana's original forest cover has been converted to anthropogenically derived, farm-bush savanna. All substantial expanses of remaining forest habitat in the country have been set aside as forest reserves, most of which are actively managed for timber harvest, and some of which have essentially no forest remaining (Hawthorne and Abu-Juam, 1995). Only about 1% of old growth forest occurs outside the boundaries of existing reserves, and sacred forest groves account for the bulk of this off-reserve forest habitat. More than 1400 sacred groves are known to occur throughout Ghana's five physiographic regions, but this number is thought to reflect only a fraction of those actually present (Tufour, et al., NCRC). Most (if not all) extant groves in the country remain as discrete patches dotting the non forest landscape matrix.

Seven forest fragments were sampled; five sacred forest groves and two large forest reserves for comparison. Fragments ranged in size from 6 to 5000 ha (Table 1) and are all centered around Kumasi (6°41'21.85" N, 1°37'24.93" W), the capital city of the Ashanti administrative region in Ghana (Figure 1). All seven fragments are representatives of the moist semi-deciduous forest habitat subtype. All are also completely surrounded by farm bush savanna, except for Owabi Forest Reserve, which adjoins the reservoir that supplies water for the city of Kumasi, and for Kajease sacred grove, where the once surrounding rural countryside is rapidly being completely supplanted by suburbanization.

Approximately 900 butterfly species are known for Ghana. The large majority of these are forest-associated species, about 1/3 of which are fruit-feeders from the family Nymphalidae. The guild of fruit-feeding forest butterflies has been beneficially exploited to study many aspects of tropical forest ecology in natural, managed, and degraded ecosystems (e.g. Kremen, 1992; Fermon et al., 2000; Stork et al., 2003; Bossart and Opuni-Frimpong, 2009; Hill et al., 2011). Member species can readily be collected via fruit-baited traps, which facilitates the systematic collection of species diversity data, and they display a wide range of relative sensitivities to habitat loss, fragmentation, and patch degradation, which makes them superb indicators of environmental change.

The fruit-feeding butterfly community of each forest fragment was sampled using typical fruit-bait traps. Traps were hung in the understory 8-10 cm above the ground. Three to four traps were installed at 80m intervals along each of two to four 'straight line' transects in each forest fragment. Each transect was established by using a compass to set direction and a meter tape to determine distance. A machete was used where necessary to gain passage through the understory. Individual transects were located in discrete areas of the forest at least 300m distant (and generally much more) from any other transect, except in the three smallest fragments where inter-transect distance was constrained by the small size and irregular shape of the forest patch. Traps were placed in similar micro habitats within areas of closed canopy forest. A total of 78 traps was installed across all sites; more traps were hung at larger sites, fewer at smaller sites (Table 1).

Trap sampling was initiated July 2005 and continued through May 2006. In general, samples were collected from each site on a 3-week rotational basis. Each sampling bout consisted of two sampling days. Traps were baited with mashed, fermenting banana, and butterflies collected the following day ~24 h later. Traps were then re-baited and left for an additional 24 h, after which trapped butterflies were again retrieved. All specimens captured in traps were retained for subsequent identification. Specimens were stored in a laboratory freezer at the Forestry Research Institute of Ghana, Fumesua, until shipment via DHL express courier to Carnegie Museum of Natural History (CMNH), Pittsburgh, Pennsylvania.

Specimens were pinned, labeled, and authoritatively determined (Dr. John Rawlins, CMNH). Project specimens are curated into a separate section of the CMNH collection, segregated by sex for each species. All data have been entered into an on-line searchable, fully relational

database, which is accessible on the Products page of the Ghana Butterfly Biodiversity project website <http://iz.carnegiemnh.org/GhanaBFly/>.

For the genetic analysis, individuals of the forest butterfly species, *Aterica galene*, *Euphaedra medon*, and *Gnophodes betsimena*, were collected from each site using a combination of fruit-baited traps and hand-held nets. These particular species were chosen because each occurs in sufficient numbers (for collection purposes) in each of the seven forest fragments and because they differ in their relative fidelity to forest habitat and predicted ability to disperse across the landscape, and consequently, their sensitivity to habitat fragmentation. Specimens were stored in 95% ethanol prior to DNA extraction. Although the goal was to collect 15 individuals per species per site for sequencing, fewer were ultimately collected at some sites (Table 1).

2.2. Molecular data

DNA was extracted from the whole abdomen of butterflies. A 710bp region of mitochondrial cytochrome oxidase I (mtDNA COI) was amplified using the primers LCO1490 (forward direction) and HCO2198 (reverse direction) (Folmer et al., 1994). PCR products were sequenced by the Genomics Core Facility at the Pennington Biomedical Research Center (Louisiana State University System, Baton Rouge, LA). Sequences were aligned and subsequently trimmed to a common length of 578bp, 570bp and 571bp, for *A. galene*, *E. medon*, and *G. betsimena*, respectively. Full details of the molecular analysis are as described in Bossart and Antwi (2013).

2.3. Diversity within sites

Species diversity was evaluated as: (a) rarefied richness, to control for differences in sample size; (b) Chao1 estimates of total richness, to estimate the total number of species predicted at each site; and (c) Simpson's (inverse) Index of Diversity, to quantify overall diversity. EstimateS 9.1.0 (Colwell, 2013) was used to calculate all measures of species diversity. Species richness values were rarefied using individual-based rarefaction to standardize sample size across sites, with all larger samples interpolated to sample sizes equivalent to those of all smaller samples (Gotelli and Colwell, 2001; Gotelli and Chao, 2013). EstimateS 9.1.0 also allows for the extrapolation of rarefied richness beyond the size of the reference sample, such that all smaller samples can additionally be extrapolated and compared to all larger samples (Colwell et al., 2012). Chao1 is a non parametric, abundance-based richness estimator that tends to be more precise and less biased than other estimators (Brose and Martinez, 2004; Walther and Moore, 2005; Hortal et. al., 2006). Simpson's Index, considered the most robust and biologically meaningful diversity index (Magurran, 2004), combines both richness and relative abundance into a single number of overall community diversity.

Genetic diversity within populations was estimated as: (a) rarefied haplotype richness; (b) percent private haplotypes; and (3) haplotype diversity, a measure of overall genetic diversity analogous to Simpson's Index of community diversity. Haplotype richness per population was determined using DnaSP v.5 (Librado and Rozas, 2009). A rarefaction calculator was then used to calculate rarefied haplotype richness, with values rarefied to a sample size of $n = 7, 14$, and 9 for *A. galene*, *E. medon*, and *G. betsimena*, respectively, across all populations; a value of 1.0 indicates a population fixed for a single haplotype. Haplotype diversity was estimated using ARLEQUIN v 3.0 (Excoffier et al., 2005), and is the probability that two randomly chosen haplotypes are different. Both observed haplotype richness and haplotype diversity data were previously reported in Bossart and Antwi (2013), but were evaluated in a different context.

Simple linear regressions were used to assess whether measures of diversity were dependent on forest fragment size, where each measure of diversity was regressed on fragment size ($\ln$ transformed). Pearson's product-moment correlations were conducted to test whether site specific measures of community diversity (e.g. species richness, Simpson's diversity) correlated

with site specific measures of genetic diversity (e.g. haplotype diversity). All regression and correlation analyses were conducted using SAS[®] 9.3 (SAS Institute Inc., Cary, NC).

2.4. Diversity across sites

Nonmetric multidimensional scaling (hereafter, NMDS; McCune and Grace, 2002), was used to visualize similarity/dissimilarity in community composition among sites. Raw data were square root transformed prior to analysis to give less weight to dominate species (Jongman et al., 1995). The initial distance matrix was calculated using the Bray-Curtis abundance based index (Legendere and Callagher, 2001; Magurran, 2004). In all cases, the algorithm was run for 200 iterations. A SIMPROF analysis, which also calculates a Bray-Curtis resemblance matrix, was conducted to investigate whether any significant internal structure was present, i.e. clustering of sites/communities based on their similarity/dissimilarity. Both NMDS and SIMPROF analyses were carried out with Primer v6 (Clarke and Gorley, 2006).

Genetic dissimilarity was calculated as the standardized measure of pairwise genetic differentiation (F'_{ST}), which is independent of within population diversity (Merriman and Hedrick 2011). The standardized form was used because pairwise F_{ST} will be low even when there is no genetic overlap between populations when intrapopulation genetic diversity is high. We used Mantel tests to statistically compare the community and genetic dissimilarity matrices to assess whether sites with the most genetic similarity were likewise those with the most similar communities and vice versa. Mantel tests were conducted with XLStat[®], Version 2014.4.10 (Addinsoft, New York, NY) using genetic distance matrices calculated separately for each species and by averaging the values for all three species (*sensu* Vellend, 2003). *P* values were generated with 1000 permutations.

Species collected from each site were also categorized according to their relative abundance, habitat association, and geographic range distribution to investigate whether sacred grove communities comprise inherently different groups of species relative to those in the large reserves. Species were assigned to categories using Larsen et al. (2007) and Belacastro and Larsen (2006), where data compiled from over a decade of research are summarized (Larsen, 2005). Species were categorized into one of four relative abundance categories: rare (RA), not rare (NR), common (CO), and very common (VC); one of five habitat association categories: wet/moist forest specialist (WET), all forest subtypes (ALF), dry forest specialist (DRY), both forest and savannah (UBQ), and savannah specialist (SAV); and one of six range distribution categories. For ease of discussion, distribution categories were simply designated 1-6, with 1 being the most narrow distribution (e.g. species endemic to the Ghana subregion; Belacastro and Larsen, 2006), 6 the broadest (e.g. species found throughout much of Africa in suitable habitat; Belacastro and Larsen, 2006), and numbers in between representing intermediate, increasingly broader distributions. Tests for association were conducted using PROC FREQ (SAS[®] 9.3; SAS Institute Inc., Cary, NC) to determine whether sacred grove communities differed from those in the forest reserves with respect to the relative frequency of species in each trait category. A Fisher's exact test, with the Monte Carlo option of estimation, was used since some trait by site categories were represented by only a few species.

3. Results

3.1. Diversity within sites

From 13-18 sampling bouts took place at each site over the course of the nearly year-long study (trap days divided by the number of traps), resulting in 115 total species from the 8164 total individuals collected (Table 1). Although estimated species richness and overall community diversity were highest at one of the large forest reserves (Owabi), estimated richness at one of the

sacred groves and total diversity at three of these small forest patches actually exceeded that of the largest forest reserve. Only a small number of generalists and savanna associated species were collected at each site. Instead, the large majority of collected species were forest habitat specialists (Figure 2a).

Cumulative rarefied richness was highest, and nearly identical, for the two large forest reserves, although the curve for the largest forest reserve (Bobiri) suggested the richness asymptote would be reached at a lower point (Figure 3). Curves were still steeply rising at most sites, including for Bonwire, a very small sacred grove, indicating that additional species were still being collected at a relatively high rate when sampling stopped. Only Gyakye and Kajease, two of the smallest sacred groves, showed evidence of an obvious decreasing rate of new species being collected. Collector's curves for all other groves fell within the 95% confidence limits of values for the largest reserve. These three sacred grove communities showed only very limited loss of species richness (4-14%) compared to the large forest reserves (Table 2). Even the two groves that showed a notable reduction, Kajease (6 ha) and Gyakye (15 ha), retained nearly 70% or greater (depending upon whether interpolated or extrapolate values are compared) of the richness found in the much larger forest reserves.

Genetic diversity was very high in both *A. galene* and *E. medon*, and extremely low in *G. betsimena* (Table 3). All *A. galene* and *E. medon* populations had haplotypes unique to a particular population, and the sacred groves generally had as many or even more private alleles than the large reserves. Interestingly, given the paucity of diversity documented for *G. betsimena*, all unique haplotypes identified for this species were associated exclusively with one of the smallest sacred groves.

Rarefied species richness increased with patch size, although this relationship was not strongly support ($p=0.016$ and $p=0.054$, for interpolated and extrapolated richness, respectively; Table 4). However, neither total estimated richness nor Simpson's Index of overall diversity were related to fragment size. All measures of genetic diversity for all species were also independent of patch area (Table 4).

Aterica galene was the only butterfly species where positive correlation between measures of species and genetic diversity was evident (Table 5). Four of the significant correlations, however, involved either observed haplotype or observed species richness, which don't account for sample size differences across sites. None of the correlations were very strong and all disappeared when Kajease, where all measures of diversity were consistently lower than those at other sites (Tables 1-3), was removed from the analysis.

3.3. Diversity across sites

Only 20 species were shared across all seven sites (Appendix 1). All sites except for Kajease, the smallest grove, had forest associated species collected only from that site. Of the 34 forest species collected from a single site, most were collected from the two large reserves (13 and 12 from Bobiri and Owabi, respectively). Most were also uncommon species represented by a single individual (63%) or at most by two individuals (17%), which means the larger number collected from the forest reserves was at least partially due to the generally larger overall samples collected from these sites. But even with this difference in sample size considered, fewer site-specific species were collected from the sacred groves.

Significant internal group structure was evident, but this clustering of communities into groups was unrelated to fragment area (Figure 4). At 65% within-group community similarity, three groups were statistically supported (Simprof; $p=0.0003$). Notably, one of these groups included both the 5000 ha forest reserve and Bonwire, the 8 ha sacred grove, but excluded, Owabi, the other large reserve, which constituted its own 'group'. Nonetheless, the main dichotomy occurred at 60% within-group similarity (Figure 4) and produced a group that included both reserves and two of the sacred groves and a second group that included the remaining three sacred groves

(Simprof; $p=0.0001$). Interestingly, these two main groups generally divided north and south of an imaginary line running east to west. In general, the greatest dissimilarities were between Kajease and the large forest reserves.

Each community comprised generally similar types of species (Figure 2). Although the community at the smallest sacred grove had significantly fewer wet forest species than the community at the largest forest reserve ($df=3$, $\chi^2=9.05$, $p=0.018$), this was the only detectable difference. These two fragment size extremes had similar frequencies of species in each of the abundance ($df=3$, $\chi^2=3.45$, $p=0.385$) and range distribution ($df=5$, $\chi^2=5.04$, $p=0.458$) categories, and no other sites differed at all. In general, the relative number of species represented in each habitat association ($df=18$, $\chi^2=10.29$, $p=1.000$), abundance ($df=18$, $\chi^2=0.930$, $p=0.977$), and range distribution ($df=30$, $\chi^2=9.85$, $p=1.000$) category was independent of site (and therefore, patch size). Consequently, sacred grove communities were not simply the subset of species most likely to withstand or even benefit from the effects of habitat fragmentation and landscape transformation, e.g. broadly distributed, abundant generalists.

There was no evidence that sites with the most dissimilar (or conversely, similar) communities were likewise those with the most dissimilar (or conversely, similar) gene pools. Pairwise community dissimilarity between forest sites was uncorrelated with pairwise genetic dissimilarity for both *A. galene* ($r_{xy}=0.194$, $p=0.393$) and *E. medon* ($r_{xy}=0.009$, $p=0.922$) considered separately, and also with genetic dissimilarity averaged across species ($r_{xy}=0.169$, $p=0.478$).

4. Discussion

Extensive expanses of undisturbed, protected habitat will continue to stand as a cornerstone of biodiversity conservation, as many of our most threatened taxa, particularly large-bodied vertebrates, have high minimum-area requirements. Ensuring the long term persistence of these species will require dedicated, ongoing investment in the preservation of large and connected tracts of intact core habitat. However, setting aside large reserves is increasingly problematic and no longer even an option in many regions as human-modified landscapes are rapidly becoming the dominant ecosystem on Earth (Goddard et al., 2010; Miller, 2007; Miller et al., 2009; Ellis and Ramankutty, 2008). Out of necessity, conservation efforts have had to increasingly rely on the protection of remnant habitat patches and other ecological elements, e.g. urban parks and gardens (Nielsen et al., 2014), that persist in transformed landscapes to piece together effective conservation strategies. Although such piece meal conservation will undoubtedly have restricted utility for protecting many species of concern, this study demonstrates that for butterflies at least, very small habitat patches can have a conservation value that rivals that of dramatically larger, gazetted and protected forest reserves. Of the multiple aspects of species and genetic diversity we quantified, only rarefied species richness followed the expected relationship between area and diversity. But even in this case, the relationship was generally weak, with the sacred groves experiencing only limited loss of species richness (e.g. generally only 4-14% lower; Table 2) despite accounting for less than 0.5-11% of the total area of the forest reserves.

The one consistent exception was Kajease, the smallest sacred grove, which had a third fewer species than the large reserves at comparable subsample sizes, harbored fewer wet forest species and no unique species, and consistently ranked lowest in nearly every other aspect of species and genetic diversity measured. Factors besides simply patch area, however, are likely responsible for the diversity decline documented in this particular grove. Kajease is the only grove that has become nearly entirely engulfed by a heavily urbanized landscape because of its proximity to what ultimately became the ever expanding city of Kumasi, a major metropolitan area with a population that now exceeds two million. Cultural lore dates this sacred grove to the early 18th century, where according to legend it was key in the Ashanti-Denkyira war because of its strategic distance from Kumasi. Notably, the traditional protective measures at Kajease are also no longer respected and degradation directly due to human intrusion and foot traffic is obvious.

Although factors affecting biodiversity in urban habitat islands are only beginning to receive attention, Lizée et al. (2102) found the major determinant of butterfly diversity to be the structure of the surrounding urban matrix, rather than park size (although see, Nielsen et al., 2014). Matrix context also determines the strength and extent of edge effects (Gaublomme et al., 2008; Laurance, 2008; Campbell et al., 2011). External pressures emanating from an urban matrix, e.g. pollution, are bound to generate greater declines in patch quality relative to those associated with non urbanized landscapes (Raupp et al., 2010), which should also heighten biodiversity loss from urban habitat patches (Franzén and Nilsson, 2010). Species diversity on habitat islands is fundamentally a balance between colonization (who gets there) and extinction (who persists there), and the extensive urbanization that has ultimately grown to surround Kajease sacred grove over time represents a formidable barrier to any colonization that might counterbalance any loss of diversity that has occurred.

Basic measures of species diversity, such as species richness, are limited in terms of what they reveal about communities because they do not account for species identities and changes in community membership, specifically, species replacements and compositional turnover. Consequently, even though we found the sacred grove communities to have nearly comparable to even higher species diversity (depending on the measure being compared) than the forest reserves, their value from a conservation perspective would be diminished if vulnerable species had generally been supplanted by cosmopolitan or ubiquitous generalists. Species that are rarer, narrowly distributed, and/or resource specialists tend to be more prone to extinction because they are less able to withstand or to recover from the pressures that promote population declines (Rabinowitz, 1981; Purvis et al., 2000; Ewers and Didham, 2006; Harnik et al., 2012). Indeed, such species-specific traits are routinely used to prioritize and list species of conservation concern, especially when data available are insufficient to determine literal imperilment or population decline (Bossart and Carlton, 2002; Knapp, 2011). The persistence of a smaller number of tolerant or generalized species that thrive in transformed landscapes (so-called 'winners') at the expense of a larger number of sensitive, habitat specific species (so-called 'losers') would also promote regional biotic homogenization and a decrease in beta diversity across the landscape as formerly dissimilar communities became more alike (McKinney and Lockwood, 1999; Clavel et al., 2010; Le Viol et al., 2012; McCune and Velland, 2013). However, we uncovered little evidence that common, broadly distributed, habitat generalists had generally become more prevalent in the sacred groves relative to their representation in the large forest reserves. Except for the somewhat lower number of wet forest specialists collected at Kajease versus Bobiri (the smallest, urban-locked site versus the largest forest reserve), the relative numbers of species in each of the abundance, habitat association, and range distribution categories were comparable across sites. Additionally, the clustering of sites, which was based on their community similarity (i.e. member species and their relative abundances), was unrelated to fragment size (Fig. 3). The community at Bonwire, for example, the second smallest grove sampled, was more similar to the largest forest reserve community than the larger reserves were to each other, or than Bonwire was to the other very small sacred groves. This unique shared similarity of Bonwire with the dramatically larger forest reserves aligns well with the earlier findings of Bossart et al. (2006).

Given that levels of species and genetic diversity in habitat patches are both influenced by relative patch size and connectivity, spatial patterns of species and genetic diversity are expected to correlate in fragmented landscapes (Vellend et al., 2014). Small, isolated patches are expected to have reduced species diversity relative to larger expanses of habitat because they are likely to support viable populations of a smaller number of species, to have less resource heterogeneity and fewer available niches, to be more degraded due to increased exposure to damaging external pressures, and simply because they tend to collect fewer species over time via passive sampling (Conner and McCoy, 1979; Saunders et al., 1991; Debinski and Holt, 2000; Robles and Ciudad, 2012). Small, isolated patches are also expected to have reduced genetic diversity (Frankham, 1995; Frankham, 1996). Because of decreased resource availability, the carrying capacity for

individual species will tend to be lower in small patches, resulting in smaller populations. With restricted inter-patch dispersal and gene flow, genetic diversity will be lost through drift and inbreeding, which can lead to further reductions in population size and additional declines in genetic diversity (a so-called, extinction vortex).

Despite these parallel expectations of reduced diversity in small patches, we found only limited evidence of positive species-genetic diversity correlations (SGDCs). This result is not really surprising given that none of the diversity measures we assessed were strongly associated with the two main drivers of diversity decline, fragment area and patch isolation. Of the three species investigated, *A. galene* was the only one for which any site-associated correlations between species diversity and haplotype diversity were detected. But even in this case, levels of significance were generally weak and largely a consequence of the reduced diversity at a single site, Kajease, the site uniquely impacted by urbanization. Sites that shared the most or least community similarity were also not the ones that shared the most or least genetic similarity. This lack of congruence between pairwise diversity matrices suggests that either different processes are shaping these two components of diversity or that one level is more sensitive to change than the other. Regardless, the absence of strong association between site-specific measures of species and genetic diversity raises an important cautionary note against relying on single elements of diversity to evaluate and prioritize the conservation value of habitat fragments. Gyakye, for example, had relatively low species diversity, but this site harbored the entirety of the unique haplotype diversity detected for *G. betsimena*.

Finding that SGDCs are apparently species-specific makes sense. The suite of ecological and life history traits that characterize a species will determine how readily habitat fragmentation creates conditions that reduce effective population sizes and promote genetic drift. Genetic consequences of fragmentation will increase with decreased connectivity among populations, and isolation is expected to be greater for species with limited dispersal ability or strict fidelity to the habitat patch (Ewers and Didham, 2006; Louy et al., 2007; Habel et al., 2009). *Aterica galene* is a relatively small butterfly that exhibits strong fidelity to forest habitat (Elbers and Bossart, 2009), and was previously shown to be the most sensitive of the three to fragmentation based on the relative extent of population genetic substructure present (Bossart and Antwi, 2013). A positive SGDC was likewise only detected for one of three bat species studied, which was again the one most sensitive to fragmentation, although sensitivity in this case was documented as a decline in allelic diversity with decreasing area (Struebig et al., 2011). Interestingly, not only did we not detect a general match-up between patch area and genetic diversity within *A. galene* populations, but this species had the most haplotype diversity overall (Table 3; also Bossart and Antwi, 2013).

So what might explain the limited loss of biodiversity from these small sacred groves? The range in area across the seven sites that were surveyed represents a sizable difference and insects additionally rapidly respond to environmental change, characteristics that should promote detection of substantial declines or compositional turnover in remnant forest patches. Indeed, with respect to species-area effects, significant positive relationships have commonly been detected for butterflies or moths (Öckinger et al., 2010), including across habitat areas that constitute only a subset of the sizes studied here but with an even smaller size differential between the smallest and largest sites (e.g. Wilcox et al., 1986; Steffan-Dewenter and Tschardt, 2000; Koh and Sodhi, 2004; Öckinger et al., 2006; Williams, 2011).

Possibly the observed levels of diversity we documented will ultimately give way to greater reductions, thereby producing clear diversity-area associations. Biodiversity loss can take many years to manifest after habitats become fragmented, creating an extinction debt that is only slowly paid out over time (Tilman et al., 1994; Keyghobadi, 2007; Kuussaari et al., 2009). When extinctions lag well behind the actual environmental perturbation, the true extent of biodiversity loss will be obscured until the new equilibrium (community or genetic) is reached.

Specific details on the history and timeframe of land cover change in Ghana are scarce, but large-scale deforestation in the country dates to at least the very early 1900s (Hawthorne and Abu-

Juam, 1995). Nearly all existing forest reserves had been established by 1939 (Dickson, 1969), and remaining forest habitat outside these was depleted at a rapid pace, particularly from 1955 to 1972 (Hawthorne and Abu-Juam, 1995). In the current landscape, Guinea savanna dominates and only scattered shreds and small patches of forest remain in the off-reserve matrix. This general setting, whereby the forest reserves stand out in sharp contrast to the surrounding matrix, has almost certainly been in place since the early 1900s, when the reserve system was established to protect any large blocks of forest that remained. Destructive wildfires that swept across the semi deciduous forest zone in the early 1980s (Amissah et al., 2010), would have further altered the extent and condition of any forest habitat remaining both within and outside of reserve boundaries, and further isolated any remaining forest fragments. Although no exact records exist, most of the country's sacred groves likely also date to at least the early 1900s, given the broad scale fragmentation that characterized the landscape. Cultural lore, however, indicates at least two of the sacred groves (Asantemanso and Kajease) have been protected for more than 200 years.

Stable communities are expected to form fairly quickly in smaller, more isolated habitat patches and for small species with rapid life cycles (Kuussaari et al., 2009), conditions which certainly describe butterflies in Ghana's sacred groves. However, present-day communities can also sometimes retain the signature of past habitat availability even many decades later (Kuussaari et al., 2009), even present-day insect communities (Sang et al., 2010). Using relative abundance as a proxy, there is little evidence that species collected from the sacred grove communities have overall smaller populations versus their counterparts in the forest reserves (Appendix A). To the extent that population decline is a forerunner of population extinction, this general lack of apparent, in-progress decline is at least consistent with an interpretation that vulnerable species have already disappeared from these communities. Given species extinction debts are challenging to establish under any circumstances (Kuussaari et al., 2009; Jackson and Sax, 2010), the general lack of detailed data on forest cover change over time for Ghana means that long term monitoring of these communities going forward will be necessary to establish whether biodiversity declines are currently pending.

The genetic data also seem incompatible with an extinction debt explanation. We previously established the presence of extensive haplotype variation in both *A. galene* (41 haplotypes) and *E. medon* (24 haplotypes), and evidence of fragmentation-associated, genetic discontinuities across the landscape, particularly for *A. galene*, the species with the highest predicted relative sensitivity to fragmentation (Bossart and Antwi, 2013). Detectable genetic substructure suggests the lack of a diversity-area relationship is not simply due to an inability of the mtDNA to track genetic changes specific to our timescale of interest. More importantly, haplotype diversity appears to have actually increased (via recent mutations) since populations of these species became fragmented (Bossart and Antwi, 2013), which means new haplotype variation is apparently being generated more rapidly than it is being lost via drift. This higher rate of diversity accumulation versus loss, in particular, runs counter to the dynamics associated with extinction lags and the processes generating positive diversity-area relationships. Nonetheless, evolutionary inferences based solely on mtDNA sequence data can be problematic given this haploid organelle's unique evolution (Galltner et al., 2009). Consequently, future analysis using a rapidly evolving nuclear marker, such as microsatellite DNA, would provide a valuable independent perspective and might reveal additional clues about the role of extinction lags (although see Habel 2014). Although mitochondrial and nuclear diversity most often show generally concordant patterns (Zink and Barrowclough, 2008; Toews and Brelsford, 2012; Bowen et al., 2014), the key question is how readily diversity loss is revealed. Such studies are few in number, but comparative analyses of mitochondrial and microsatellite diversity in historical versus extant gene pools actually suggest that extinction lags are less likely to be associated with mitochondrial polymorphisms owing to the faster rate of gene loss (Mondol, 2013; Pacioni et al., 2015; Xenikoudakis et al., 2015).

In general, though, failure to detect strong diversity-area relationships does not have to mean that the effects of fragmentation have yet to materialize or are otherwise obscured by

528 confounding factors. Discrepancies from expected relationships for terrestrial habitat islands are
529 not uncommon and fragment size can be a poor predictor of both species (Debinski and Holt,
530 2000; Ewers and Didham, 2006; Prevedello and Vieira, 2010) and genetic diversity (Keyghobadi,
531 2007). Unlike classic island biogeography theory, which dichotomizes the landscape into habitat
532 islands and a wasteland sea, the landscape matrix surrounding terrestrial habitat fragments is not
533 necessarily an inherently hostile environment, completely devoid of resources for forest associated
534 species. Rather, it is a dynamic and complex mosaic of vegetative communities and environmental
535 features, where relative matrix permeability (and hence, connectivity of populations) depends on
536 matrix composition and structure, and on species-specific perceptions and dispersal abilities (Lord
537 and Norton, 1990; Ricketts, 2001; Debinski, 2006; Taylor et al., 2006; Prevedello and Vieira, 2010).
538 It is now widely appreciated that the matrix can mitigate biodiversity loss from habitat patches by
539 providing additional resources that help maintain populations within patches, and by facilitating
540 dispersal and connectivity between patches (Ricketts, 2001; Bender and Fahrig, 2005; Debinski,
541 2006). Distance between pairwise populations did not explain either community or genetic
542 similarity/dissimilarity among the sites we studied, which is one pattern predicted if the surrounding
543 landscape has played a key role in maintaining within patch diversity. However, simple Euclidean
544 distance is also unlikely to capture the interplay between landscape pattern and patch diversity.
545 Regardless, whatever landscape factors might be potentially at work, their effects on species and
546 genetic diversity are seemingly acting independently given the lack of correlation between the
547 pairwise diversity matrices. An important focus for future studies will be to investigate explicitly,
548 using recent analytical and technological advances for assessing landscape pattern, to what extent
549 the surrounding matrix has influenced sacred grove gene pools and communities.

550 A third, and perhaps the most hopeful, explanation for the limited loss of biodiversity we
551 documented is that the customary laws enforced by local communities have been broadly effective
552 at mitigating the negative effects of the small size of these groves. Gaining research permission
553 from village elders and chiefs to enter and collect from each grove, spanned from a single, one-on-
554 one visit with the village chief, to many visits with elders, chiefs, and communities that involved
555 negotiations, ceremonies, and the pouring of libations. Not surprisingly, the sites where obtaining
556 permission was difficult and involved (Asantemanso, Bonwire, and Kona), ultimately proved to be
557 the most intact with essentially no to minimal evidence of direct human impact. For these forests,
558 protective measures were clearly still strongly in place and respected by members of the local
559 community. Access to Bonwire was further restricted by the 45 minute walk required to reach the
560 grove from the village. Conversely, where permission was easily and quickly gained (Kajease and
561 Gyakye), the forests showed multiple signs of disturbance due to human activities and
562 encroachment. For these two forests, any customary protections that remain are only loosely held
563 at best. Although we found no obvious consequences for genetic diversity, levels of species
564 diversity across groves broadly separated according to these levels of protection and impacts, with
565 diversity at Asantemanso, Bonwire, and Kona significantly more aligned with that at the large
566 reserves than with either Kajease or Gyakye. This same separation among sacred groves
567 according to levels of protection was evident in the earlier study as well (Bossart et al., 2006).

568 Community-based conservation strategies have often proved unsuccessful, commonly
569 because of misalignment between the desires and priorities of communities and the goals and
570 objectives of conservation agencies (Berkes, 2004; Mulrennan, 2012). Failures often result when
571 initiatives are designed, developed, and implemented by external institutions and experts, with little
572 actual local participation and devolution of control (Kellert et al., 2000; Campbell and Vainio-
573 Mattila, 2003; Berkes, 2004). The community-based measures that protect sacred groves,
574 however, are specifically founded on the shared values of local residents and authorities, with
575 locally appointed and respected chiefs and elders overseeing management and setting regulations
576 and penalties. This tight link with the local residents can help promote long-term sustainability of
577 community-based reserves (Lee and Schaaf, 2003), particularly when the cultural measures in
578 place are strong and have been enforced over a long time period.

How broadly generalizable our result of limited biodiversity loss proves to be across different taxonomic groups and ecological settings remains to be established. Clearly, many of Ghana's forest associated species will not benefit from protection of the country's sacred groves because their resource needs exceed what the groves can supply, e.g. space. For many large-bodied species that tend to occur at low densities, small scattered islands of old-growth forest will never replace the need for large blocks of formally protected forest habitat. But for many of *the little things that run the world* (Wilson, 1987) and even for many of the somewhat larger *things*, e.g. small mammals and birds, a well-protected grove may harbor much, or even more, of the biodiversity found in large governmental reserves. In this context alone, sacred groves will be a critical element of any effective conservation management strategy. But this value becomes even more magnified in countries like Ghana, where sacred groves account for nearly all remaining patches of off-reserve forest habitat.

The status of most of Ghana's many sacred groves is unknown. Many are yet to be officially identified and nearly all remain unstudied. Of the groves studied here, time for action has undoubtedly expired for Kajease, which has been reduced to a thin shred of habitat as a consequence of continual encroachment (Google Earth, Image © CNES/Astrium, 1/14/2105, eye alt 7000 ft). The significant challenge will be to implement official national and international initiatives that strengthen and safeguard traditional protective measures where opportunity still exists without simultaneously eroding community support and alienating local residents.

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Table 1

Site specifics, trap collection data, and community diversity summary statistics. Number of individuals collected (N_s), number of species collected (Obs_{Sp}), Chao1 estimate of total richness (Est_{Sp}), Simpson's Index of overall species diversity (D_{Sp})

Site	Coordinates	Size (ha ²)	Traps	Trap Days ^a	N_s	N_s per trap day	Obs_{Sp}	Est_{Sp}	D_{Sp}
Bobiri	6°42' N, 1°15' W	5000	16	224	1292	5.77	67	80.32	13.84
Owabi	6°44' N, 1°41' W	1200	16	288	2445	8.49	80	105.66	19.86
Kona	6°52' N, 1°31' W	130	12	168	1323	7.88	61	103.97	16.90
Asantemanso	6°28' N, 1°33' W	100	11	154	1059	6.88	60	73.32	18.32
Gyakye	6°33' N, 1°31' W	11.5	8	112	752	6.71	47	54.49	10.62
Bonwire	6°46' N, 1°28' W	8	8	128	698	5.45	49	72.97	15.48
Kajease	6°38' N, 1°39' W	6	7	91	595	6.54	37	53.47	8.42

^aTrap days is calculated as the number of traps multiplied by the number of sampling bouts at the site.

Table 2

Rarefied species richness for standardized subsamples at each forest patch site. Sites are listed from smallest to largest samples collected. Numbers above the diagonal are rarefied values for larger samples interpolated to the subsample size of the smaller sample. The smallest sample (596), for example, was collected from Kajease. The rarefied richness value for Bonwire (where a larger overall sample was collected) interpolated to the subsample size of 596 is 47. Numbers below the diagonal are rarefied values from smaller samples extrapolated to the sample size of all larger samples. The rarefied richness value for Kajease, for example, extrapolated to the larger sampled collected from Bonwire is 39. Numbers in parentheses are 95% confidence intervals.

Abbreviations are as in Table 1.

Site	Trap Data	Rarefied Richness Values						
		Kajease	Bonwire	Gyakyee	Asantemanso	Bobiri	Kona	Owabi
Kajease – 6ha	Obs _{Sp} =37 N _s =596	--	47 (38-55)	45 (39-50)	51 (44-57)	55 (48-62)	48 (39-57)	54 (46-63)
Bonwire – 8ha	Obs _{Sp} =49 N _s =698	39 (31-47)	--	46 (41-52)	53 (47-60)	57 (50-64)	51 (41-60)	57 (48-65)
Gyakyee – 15ha	Obs _{Sp} =47 N _s =752	40 (32-48)	50 (41-59)	--	55 (48-61)	58 (52-65)	52 (42-61)	58 (49-66)
Asantemanso – 100ha	Obs _{Sp} =60 N _s =1059	45 (34-55)	58 (47-69)	50 (44-57)	--	64 (57-71)	58 (48-68)	64 (55-72)
Bobiri – 5000ha	Obs _{Sp} =67 N _s =1292	48 (35-60)	60 (48-72)	52 (45-60)	63 (56-71)	--	61 (51-72)	67 (59-76)
Kona – 130ha	Obs _{Sp} =61 N _s =1323	48 (35-60)	61 (48-73)	52 (45-60)	64 (56-71)	67 (60-74)	--	68 (59-76)
Owabi – 1200ha	Obs _{Sp} =80 N _s =2445	56 (33-79)	72 (49-95)	56 (43-69)	72 (59-84)	76 (66-87)	77 (62-92)	--

Table 3

Genetic diversity data for *A. galene*, *E. medon*, and *G. betsimena* at each site. Haplotype richness (Obs_{Hap}), rarefied haplotype richness (RA_{Hap}), percent private haplotypes ($\%PHap$), haplotype diversity (D_{Hap}).

	N	Obs_{Hap}	RA_{Hap}	$\%PHap$	D_{Hap}
<i>A. galene</i>					
Bobiri	15	9	5.48	56	0.914
Owabi	15	12	6.40	50	0.971
Kona	15	12	6.40	58	0.971
Asantemanso	15	9	5.13	33	0.876
Gyakye	12	8	5.57	50	0.924
Bonwire	7	5	5*	60	0.857
Kajease	12	5	3.60	40	0.667
<i>E. medon</i>					
Bobiri	15	6	5.80	17	0.819
Owabi	15	8	7.67	50	0.876
Kona	15	7	6.67	28	0.800
Asantemanso	15	5	4.80	40	0.714
Gyakye	15	7	6.73	43	0.781
Bonwire	14	9	9*	56	0.943
Kajease	15	6	5.87	17	0.771
<i>G. betsimena</i>					
Bobiri	15	2	1.60	0	0.133
Owabi	14	2	1.64	0	0.143
Kona	9	1	1*	0	0.000
Asantemanso	15	2	1.60	0	0.133
Gyakye	15	5	3.40	60	0.476
Bonwire	13	1	1	0	0.000
Kajease	15	1	1	0	0.000

Table 4

Regression statistics for tests of the relationship between fragment area and measures of community and genetic diversity. Abbreviations are as in Table 3.

Diversity Measures	<i>F</i>	<i>P</i>
Community Diversity		
Interpolated richness ¹	12.92	0.0156
Extrapolated richness ²	6.26	0.0543
Chao1 richness	3.93	0.1042
Simpson's diversity	2.05	0.2116
Genetic Diversity ³		
<i>A.galene</i> RA _{Hap}	2.62	0.1667
<i>A.galene</i> % _{Hap}	0.17	0.6958
<i>A.galene</i> D _{Hap}	2.57	0.1701
<i>E.medon</i> RA _{Hap}	0.25	0.6359
<i>E.medon</i> % _{Hap}	0.26	0.6305
<i>E.medon</i> D _{Hap}	0.01	0.9459
<i>G.betsemina</i> RA _{Hap}	0.02	0.9047
<i>G.betsemina</i> % _{Hap}	0.68	0.4457
<i>G.betsemina</i> D _{Hap}	0.00	0.9693

¹Regression of interpolated rarefied richness values (interpolated to the subsample size of the smallest sample).

²Regression of extrapolated rarefied richness values (extrapolated to the sample size of the largest sample).

³Species are listed in order of their decreasing sensitivity to forest fragmentation (after Bossart and Antwi (2013)).

Table 5

Pearson product-moment correlations between measures of species and genetic diversity for three butterfly species that differ in their sensitivity to forest fragmentation. Rarefied species richness interpolated to the smallest sample size (Int_{Sp}), Rarefied species richness extrapolated to the largest sample size (Ext_{Sp}). All other abbreviations are as in Tables 1 and 3.

	Obs _{Hap}	RA _{Hap}	% _{Hap}	D _{Hap}
<i>A. galene</i>				
Obs _{Sp}	0.829*	0.789*	0.191	0.760*
Int _{Sp}	0.685	0.750*	0.235	0.797*
Ext _{Sp}	0.660	0.686	0.371	0.656
Est _{Sp}	0.833*	0.806*	0.400	0.705
D _{Sp}	0.680	0.702	0.089	0.683
<i>E. medon</i>				
Obs _{Sp}	0.098	0.047	0.198	0.199
Int _{Sp}	0.084	0.049	0.288	0.220
Ext _{Sp}	0.224	0.197	0.202	0.378
Est _{Sp}	0.288	0.236	0.164	0.319
D _{Sp}	0.201	0.169	0.500	0.231
<i>G. betsimena</i>				
Obs _{Sp}	-0.050	-0.037	-0.318	0.000
Int _{Sp}	0.058	0.066	-0.226	0.104
Ext _{Sp}	-0.442	-0.433	-0.619	-0.405
Est _{Sp}	-0.376	-0.365	-0.488	-0.345
D _{Sp}	-0.255	-0.246	-0.445	-0.217

* $P < 0.05$.

Fig. 1. Map of the seven forest fragments: 2 forest reserves (dark gray) and 5 sacred forest groves, in relation to the greater Kumasi metropolitan region. White lines indicate major roads. Developed/urban areas are light white-gray. All sites are located in the moist semi-deciduous forest zone in the Ashanti region of Ghana. Map markers point to each sacred grove. Inset shows Ghana's forest reserves, which stand out as dark shaded areas against the surrounding farm bush savanna. For orientation, Owabi (left) and Bobiri (right) reserves are circled on the inset. Inset image: Google Earth™, Image Landsat, 4/9/2103, eye alt 400 mi; Large image: Google Earth™, Image © CNES/Astrium, 1/14/2105, eye alt 50 mi. Table 2 lists coordinate positions for each site, which can be used to zoom in to their general location in Google Earth.

Fig. 2. Ecological traits of species collected at each site. Bars show the relative proportion of species within each trait category at each site. Habitat associations (A), relative abundances (B) and geographic range distributions (C). Sites are listed from largest to smallest, left to right. Abbreviations are described in the text.

Fig. 3. Rarefied species accumulation curves for each forest site surveyed. Dotted lines are 95% confidence intervals associated with Bobiri, the largest forest reserve. Sites are listed from largest to smallest, top to bottom. FR=Forest Reserve, SG=Sacred Grove.

Fig. 4. Non-metric multidimensional (NMDS) scaling ordinations of butterfly community similarity among forest sites. The solid and dotted lines indicate the average similarity shared among communities.

Appendix 1. The number of individuals collected of each species at each forest site and the category they were assigned to for each of three ecological traits. Asante=Asantemanso. Abbreviations are described in the text.

Species	Bobiri	Owabi	Kona	Asante	Gyakye	Bonwire	Kajease	Range	Rarity Class	Habitat
<i>Amauris niavius</i>	0	1	1	0	0	0	0	6	CO	GUI
<i>Amauris tartarea</i>	0	1	0	0	0	0	0	5	NR	ALF
<i>Andronymus hero</i>	1	0	0	0	0	0	0	4	NR	MEF
<i>Anthene locuples</i>	0	0	0	1	0	0	0	3	RA	WEF
<i>Anthene rubricinctus</i>	0	0	0	0	1	0	0	4	CO	MEF
<i>Ariadne enotrea</i>	2	1	1	0	0	0	0	4	VC	ALF
<i>Aterica galene</i>	25	76	17	35	9	17	14	5	CO	ALF
<i>Bebearia absolon</i>	36	11	3	7	0	3	2	4	CO	ALF
<i>Bebearia barce</i>	0	0	0	4	0	0	0	4	RA	WEF
<i>Bebearia cocalia</i>	0	4	5	0	0	0	4	4	CO	ALF
<i>Bebearia demetra</i>	1	0	0	0	0	0	0	3	RA	WEF
<i>Bebearia lucayensis</i>	2	0	1	0	2	0	0	2	RA	MEF
<i>Bebearia mandinga</i>	2	4	1	2	0	1	1	4	CO	ALF
<i>Bebearia mardania</i>	1	22	11	10	0	3	0	3	CO	ALF
<i>Bebearia oxione</i>	1	5	0	2	0	1	0	4	NR	MEF
<i>Bebearia paludicola</i>	0	2	1	3	0	0	2	3	NR	MEF
<i>Bebearia phantasina</i>	7	0	0	0	0	0	0	2	CO	ALF
<i>Bebearia sophus</i>	16	128	25	60	11	6	9	4	CO	ALF
<i>Bebearia tentyrus</i>	127	15	23	1	11	1	0	3	CO	MEF
<i>Bebearia zonara</i>	31	3	8	0	1	1	0	4	CO	MEF
<i>Bicyclus abnormis</i>	237	231	111	4	0	28	0	1	NR	WEF
<i>Bicyclus dorothea</i>	0	15	15	10	7	0	21	3	VC	ALF
<i>Bicyclus funebris</i>	149	143	127	48	21	80	21	4	CO	DRF
<i>Bicyclus madetes</i>	25	120	87	63	53	65	7	4	NR	MEF
<i>Bicyclus martius</i>	33	66	166	47	42	77	3	4	CO	MEF
<i>Bicyclus procora</i>	8	53	0	0	1	1	0	4	NR	WEF
<i>Bicyclus safitza</i>	2	12	2	3	3	1	12	6	NR	GUI
<i>Bicyclus sandace</i>	2	37	27	69	39	4	50	5	VC	ALF
<i>Bicyclus sangmelinae</i>	5	31	0	1	0	0	0	4	NR	WEF

<i>Bicyclus taenias</i>	17	43	22	30	39	10	1	4	CO	MEF
<i>Bicyclus vulgaris</i>	62	71	66	106	84	23	87	5	VC	ALF
<i>Bicyclus xeneas</i>	19	7	12	0	0	2	0	5	NR	ALF
<i>Bicyclus zinebi</i>	4	38	48	3	3	42	6	1	NR	ALF
<i>Catuna crithea</i>	0	2	0	0	0	0	0	3	VC	ALF
<i>Celaenorrhinus galenus</i>	0	1	0	1	2	16	0	6	CO	ALF
<i>Celaenorrhinus meditrina</i>	0	0	0	2	0	0	0	4	RA	WEF
<i>Charaxes ameliae</i>	0	0	1	0	0	0	0	4	NR	ALF
<i>Charaxes anticlea</i>	1	1	0	1	0	0	0	4	NR	ALF
<i>Charaxes bipunctatus</i>	0	0	1	2	6	1	1	4	NR	WEF
<i>Charaxes boueti</i>	0	2	0	0	0	0	0	3	NR	DRF
<i>Charaxes brutus</i>	1	1	1	0	0	0	0	5	CO	MEF
<i>Charaxes cynthia</i>	8	4	3	2	5	4	0	4	CO	ALF
<i>Charaxes etheocles</i>	1	0	0	0	0	0	0	4	CO	ALF
<i>Charaxes fulvescens</i>	0	0	0	4	1	2	1	5	NR	ALF
<i>Charaxes lucretius</i>	1	0	0	1	1	0	0	4	CO	ALF
<i>Charaxes numenes</i>	2	0	0	1	3	0	3	4	NR	ALF
<i>Charaxes paphianus</i>	0	0	0	0	0	1	0	4	NR	WEF
<i>Charaxes pleione</i>	0	1	0	0	0	0	0	5	CO	ALF
<i>Charaxes protoclea</i>	4	0	4	3	0	4	0	5	CO	ALF
<i>Charaxes subornatus</i>	0	1	0	0	0	0	0	4	RA	WEF
<i>Charaxes tiridates</i>	0	1	1	1	2	0	2	4	CO	ALF
<i>Charaxes varanes</i>	0	0	0	1	0	0	0	6	CO	GUI
<i>Charaxes zingha</i>	0	3	1	0	0	1	0	4	NR	MEF
<i>Cymothoe caenis</i>	0	0	3	4	5	0	1	4	CO	ALF
<i>Cymothoe egesta</i>	16	1	0	2	0	0	0	4	CO	MEF
<i>Cymothoe fumana</i>	4	0	0	0	0	0	0	3	CO	MEF
<i>Cymothoe mabillei</i>	3	12	19	0	0	5	0	3	CO	MEF
<i>Elymniopsis bammakoo</i>	17	49	36	14	9	10	16	1	CO	ALF
<i>Euphaedra ceres</i>	15	67	21	47	17	14	20	3	CO	ALF
<i>Euphaedra diffusa</i>	0	1	1	3	3	0	1	4	NR	DRF
<i>Euphaedra edwardsii</i>	0	7	1	4	0	1	1	4	CO	ALF
<i>Euphaedra eupalus</i>	4	62	0	4	3	0	0	1	RA	WEF

<i>Euphaedra harpalyce</i>	15	60	14	34	15	26	26	4	VC	ALF
<i>Euphaedra hebes</i>	3	0	0	0	0	0	0	2	NR	WEF
<i>Euphaedra inanum</i>	4	0	0	0	0	0	0	1	RA	MEF
<i>Euphaedra janetta</i>	4	8	2	0	1	0	0	3	CO	ALF
<i>Euphaedra medon</i>	45	182	111	91	67	72	49	4	CO	ALF
<i>Euphaedra modesta</i>	2	0	0	0	0	0	0	1	NR	WEF
<i>Euphaedra phaethusa</i>	60	160	39	81	18	27	16	1	CO	ALF
<i>Euphaedra themis</i>	24	68	18	14	3	24	15	2	NR	DRF
<i>Euphaedra xypete</i>	1	0	0	0	0	0	0	3	CO	MEF
<i>Euriphene amicia</i>	4	0	0	0	0	0	0	4	NR	MEF
<i>Euriphene ampedusa</i>	9	23	3	3	3	10	0	2	NR	ALF
<i>Euriphene aridatha</i>	8	37	6	1	0	1	0	3	NR	MEF
<i>Euriphene barombina</i>	25	44	18	59	16	20	0	4	VC	ALF
<i>Euriphene gambiae</i>	20	0	0	0	0	0	0	4	CO	ALF
<i>Euriphene simplex</i>	37	13	43	65	24	5	0	1	NR	WEF
<i>Euryphura chalcis</i>	0	2	0	0	0	0	0	4	CO	ALF
<i>Eurytela dryope</i>	0	3	1	2	8	1	1	5	NR	DRF
<i>Euxanthe eurinome</i>	0	0	1	0	1	0	1	4	NR	MEF
<i>Gnophodes betsimena</i>	76	286	90	63	176	53	160	6	CO	ALF
<i>Gnophodes chelys</i>	22	67	36	9	12	9	8	6	CO	ALF
<i>Gretna waga</i>	0	1	1	1	0	1	0	4	CO	ALF
<i>Harma theobene</i>	5	16	32	0	0	5	0	4	CO	MEF
<i>Hypolimnias antheson</i>	0	1	0	0	0	0	1	5	CO	ALF
<i>Hypolimnias salmacis</i>	0	0	0	1	2	0	0	4	CO	MEF
<i>Hypolycaena antifaunus</i>	1	3	0	0	0	0	0	4	NR	MEF
<i>Hypolycaena dubia</i>	4	15	5	8	0	0	0	4	CO	ALF
<i>Hypolycaena kakumi</i>	1	2	2	0	1	0	0	3	CO	MEF
<i>Hypolycaena scintillans</i>	0	7	1	1	0	2	0	3	CO	ALF
<i>Junonia cymodoce</i>	0	1	0	0	0	0	0	4	NR	MEF
<i>Junonia terea</i>	0	3	0	0	0	0	1	4	VC	ALF
<i>Lachnoptera anticlia</i>	1	0	0	0	0	0	0	4	CO	MEF
<i>Melanitis leda</i>	17	50	16	11	13	12	28	6	CO	UBQ
<i>Melanitis libya</i>	0	8	0	1	1	0	1	6	NR	UBQ

<i>Neptidopsis ophione</i>	1	2	0	0	0	0	0	5	CO	ALF
<i>Neptis melicerta</i>	0	1	1	0	0	0	0	4	CO	MEF
<i>Neptis metella</i>	1	2	1	0	0	0	0	4	CO	ALF
<i>Neptis nemetes</i>	0	1	1	0	0	0	0	4	CO	ALF
<i>Neptis nicoteles</i>	0	0	1	0	0	0	0	4	CO	MEF
<i>Neptis nysiades</i>	0	0	2	0	0	0	0	3	NR	MEF
<i>Oxylides faunus</i>	1	0	0	0	0	0	0	4	CO	MEF
<i>Palla decius</i>	1	10	5	4	4	2	3	4	NR	MEF
<i>Palla publius</i>	0	1	0	0	0	0	0	4	NR	MEF
<i>Palla ussheri</i>	6	0	0	0	2	0	0	4	CO	ALF
<i>Palla violinitens</i>	0	1	0	0	0	0	0	4	NR	MEF
<i>Papilio cyproeofila</i>	0	1	0	0	0	0	0	4	CO	MEF
<i>Pseudacraea eurytus</i>	0	2	0	1	1	0	0	4	CO	ALF
<i>Pseudacraea lucretia</i>	2	0	0	2	0	1	0	6	CO	ALF
<i>Pseudacraea semire</i>	0	1	0	0	0	0	0	4	CO	ALF
<i>Pseudoneptis bugandensis</i>	0	5	0	0	0	1	0	4	CO	ALF
<i>Pteroteinon caenira</i>	0	0	0	0	0	1	0	4	CO	ALF
<i>Salamis cacta</i>	0	1	0	1	0	0	0	5	CO	MEF
<i>Triclema hades</i>	0	1	0	0	0	0	0	4	NR	MEF

Response to the Reviewer Comment:

The one minor requested revision concerned Figure 1, which identifies the spatial orientation of the study sites. This figure was included in the previous revision at the request of one of the original reviewers. A reviewer has now expressed concern that the sacred grove outlines cannot be detected in this Figure.

While I appreciate the concern regarding background clarity, the map was only intended to show the spatial nature of the study area/sites as originally requested by the earlier reviewer. The sacred groves are too small for their boundaries to show up at the level of resolution needed to ensure all seven sites are encompassed by the map. However, to address this general concern, I took two approaches: I attempted to enhance clarity on the existing 'Google Earth' figure and I created a replacement 'cartoon map' figure. I will leave it up to the editor to decide which one is the preferred Figure 1.

My personal preference for Figure 1 is the version with Google Earth images because it shows a much more realistic depiction of existing forest cover and urbanization. For example, although insets in both Figure versions show the forest reserves, many of the reserves depicted in the cartoon version (e.g. the northernmost reserves) don't actually have any forest cover remaining, which is apparent when compared to the Google Earth inset. Hence, the cartoon map gives a false impression of existing forest cover. The cartoon map also doesn't capture the extent of urbanization that is the greater Kumasi metropolitan area. Finally, unlike cartoon maps, one can zoom in and out on Google Earth locations, e.g. using spatial coordinates to orient.

Changes I've made to address Figure 1 concerns:

- The backgrounds of the Google Earth images have been lightened to increase contrast. A different type of map marker has been used to identify sites and these have all been enlarged and outlined for better contrast. Font size has been increased. Some major roads have been included to enhance spatial orientation. The distance scale has been outlined against a white background.
- An additional line has been added to the Figure caption to remind readers they can use site coordinates (listed in Table 1) to zoom in on Google Earth to get a better sense of the surrounding landscape. Because I don't have permission from local villagers to provide exact localities of the sacred sites, for ethical reasons only the general location of each has been provided, i.e. seconds are not included. That said, one can readily gain a sense of background features (and likely locate the sacred groves) by zooming in and moving across the landscape.
- An alternate/replacement Figure 1 and caption have been included as a separate document.

A map of Ghana with the city of Kumasi marked by a star and labeled. The map shows the coastline of the Gulf of Guinea to the south and the borders with Ivory Coast to the west and Togo to the east. The interior of Ghana is shaded with a stippled pattern.

Fig. 1. Map of the seven forest fragments: 2 forest reserves (Bobiri and Owabi) and 5 sacred forest groves, in relation to the greater Kumasi metropolitan region. All sites are located in the moist semi-deciduous forest zone in the Ashanti Region of Ghana. Inset shows Ghana's forest reserves (black) and forest zones (bands of decreasing shades of gray). For reference, inset reserves closest to Kumasi are Owabi (left) and Bobiri (right).