Contrasting effects of host tree isolation on population connectedness in two tropical epiphytic bromeliads

Autumn A. Amici[1,3] (iD) , Nalini M. Nadkarni[1], Emily DiBlasi[2], and Jon Seger[1]

PREMISE: Conversion of primary forests to pastures is a major cause of habitat fragmentation in the tropics. Fragmentation is expected to impede gene flow for many plant species that are restricted to remaining forest fragments. Epiphytes may be especially vulnerable to this effect of forest fragmentation because they depend on host trees. However, trees that remain in pastures may enhance connectivity across the landscape for epiphyte species that can thrive on such trees. To investigate this possibility, we studied the genetic structures of two such species on isolated pasture trees and surrounding forest, in relation to their local abundances in different habitat types and aspects of their reproductive biology including pollen and seed dispersal agents, and looked for evidence of increased or diminished gene flow.

METHODS: We used microsatellite markers to assess geographic patterns of genetic diversity and differentiation in two epiphytic bromeliads, *Catopsis nitida* and *Werauhia tonduziana,* in the Monteverde region of Costa Rica.

RESULTS: About 85% of the F_{ST} value for *Catopsis nitida* was found among pastures within regions, while for *Weruahia tonduziana,* about 80% of the F_{ST} value was contributed by differences between regions, indicating much more gene flow within regions, relative to *C. nitida*.

CONCLUSIONS: Although there was substantial genetic differentiation among epiphyte populations, those on isolated pasture trees were not substantially less diverse than those in adjacent forests, suggesting that pasture trees may serve as "stepping stones" that help these species maintain their genetic connectedness and diversity at larger geographic scales.

KEY WORDS Bromeliaceae; *Catopsis nitida*; Costa Rica; epiphyte; fragmentation; gene flow; *Werauhia tonduziana*.

Habitat fragmentation often interrupts historic patterns of gene flow among plant populations, which increases their genetic isolation and decreases their effective population sizes (Hamrick, 2010; Allendorf et al., 2013). Smaller populations experience higher rates of genetic drift and are therefore expected to maintain lower levels of diversity (Gonzalez-Astorga et al., 2004). The International Union for the Conservation of Nature (IUCN) recognizes preserving genetic diversity as a component of biodiversity conservation (Allendorf et al., 2013). In theory, reduced genetic diversity may hinder a species' ability to evolve in response to climate change and local landscape-level processes (Allendorf et al., 2013). Thus, it is important to understand how patterns of genetic diversity vary geographically, and how they change over time in response to human alteration of landscapes.

Conversion of forest to pasture has been a major cause of habitat fragmentation in the neotropics (Holl et al., 2000; Harvey et al., 2004; Manning et al., 2006). Over time, once-intact forests become a matrix of pastures, agricultural land, secondary forest, and residual patches of primary forest (Guevara et al., 1998; Harvey et al., 2004; Manning et al., 2006; Cascante-Marín et al., 2009; Laurance et al., 2014). At the turn of the 21st century, pastures dominated roughly 45% of Costa Rica's landscape (Holl et al., 2000). Many such pastures contain isolated trees. Landowners keep trees in pastures for a variety of reasons including their service as living fence posts, for fruit products, shade and forage for cattle, for timber and firewood, and as aesthetic features that potentially increase the property's economic value (Guevara et al., 1998; Harvey et al., 2004).

Epiphytes may be especially vulnerable to deforestation as they live on trunks, branches, and in the canopy soil of trees (Gonzalez-Astorga et al., 2004; Larrea and Werner, 2010; Trapnell et al., 2013). Forest fragmentation is expected to disrupt their genetic connectedness at multiple scales across a landscape (Gonzalez-Astorga et al., 2004; Larrea and Werner, 2010; Trapnell et al., 2013) by altering pollen movement, seed dispersal, and establishment success (Hamilton, 1999; Bittencourt and Sebbenn, 2007; Gaino et al., 2010). As host trees become more isolated, epiphyte populations may become more genetically differentiated and less genetically diverse (Young et al., 1996; Bittencourt and Sebbenn, 2007; Gaino et al., 2010; Aavik et al., 2014; Finlay et al., 2017). Isolated trees in pasture habitats have been shown to have reduced epiphyte species diversity (Flores-Palacios and García-Franco, 2004, 2008) compared to intact and primary forests. In addition, epiphytes that maintain populations on isolated pasture trees may be vulnerable to inbreeding depression caused by reduced gene flow, reduced pollinator activity, limited seed dispersal beyond the originating tree, lower seed germination success, and mating among close relatives (Ishihama et al., 2005; Cascante-Marín et al., 2006a, b; Victoriano-Romero et al., 2017).

The consequences of isolation for host-tree "island" populations of epiphytes may be more complex than for members of the same species living in neighboring forest fragments (Aldrich and Hamrick, 1998; Guevara et al., 1998; Holl et al., 2000; Werner et al., 2005; Prevedello et al., 2018). Isolated remnant trees may provide links by serving as "stepping stones" or "regeneration nuclei" for flora and fauna across a discontinuous landscape (Aldrich and Hamrick, 1998; Guevara et al., 1998; Holl et al., 2000; Werner et al., 2005; Prevedello et al., 2018). For example, isolated remnant pasture trees in Mexico could still reproduce and thereby contribute to the ecological resilience of the fragmented tropical rain forest (Guevara et al., 1998; Hamrick, 2010). Pollen movement and seed dispersal can occur over long distances and can allow for continued gene flow despite fragmentation of habitat (Dick, 2001; Trapnell and Hamrick, 2005). While some epiphytes are rare on isolated pasture trees, other species are abundant, illustrating different population responses to isolation. However, the genetic consequences of isolation on pasture trees have been little studied.

Subdivision of an epiphyte population through fragmentation of its habitat may be less problematic if the resulting subpopulations are able to maintain sufficient levels of gene flow. However, where populations are both small and highly isolated, there may be negative consequences for individuals and local populations even where the genetic diversity of the species remains high at larger geographic scales (Hamrick, 2010). These observations raise an intriguing question as to whether epiphytes on isolated trees in pastures primarily experience decreased gene flow and increased inbreeding, or whether they may be playing key roles in dispersing their species and maintaining its genetic connectedness in a heterogeneous and dynamic habitat matrix.

We studied the population genetic structure of two species of epiphytic bromeliads [*Catopsis nitida* (Hook.) Griseb. and *Werauhia tonduziana* (L.B.Sm.) J.R.Grant] in forest and pasture trees in a tropical montane landscape at Monteverde, Costa Rica. Bromeliaceae is an appropriate epiphytic group for such a study because it includes species that occupy and are often abundant in these habitat types (Zanella et al., 2012; Cascante-Marín et al., 2014). Given the reduced epiphyte species diversity found by previous studies of isolated pasture trees, we hypothesized that there might be reduced gene flow and increased differentiation in both species, among pasture

populations and between habitat types. We also anticipated that patterns of genetic diversity and structuring in these species could be affected by differences in their reproductive biology (Table 1). Thus our central question was, what could we infer about patterns of gene flow and connectedness in these two species by comparing their patterns of genetic diversity in different habitat types and at different geographic scales?

MATERIALS AND METHODS

Study site

Our research site was located in the tropical rain forest habitat in the Monteverde region in northwestern Costa Rica. This area encompasses the Continental Divide on both sides down to 700 m in elevation (Nadkarni and Wheelwright, 2000). The Monteverde region is a patchwork of active pastures, primary forest, and secondary forest (Nadkarni and Wheelwright, 2000). We sampled the two bromeliad species from trees in primary forest (two sites, which have experienced no impact from land development or harvesting), mixed forest (four sites, which have experienced some forest harvesting and development), and pastures (14 sites; Fig. 1). We use the term "population" to indicate all conspecific samples at a primary forest, mixed forest or pasture study site. We grouped the populations into three regions (northern, central, and southern) within the study area for analyses of geographic differentiation (Fig. 1). Locality and GPS coordinates for each population are described in Appendix S1.

Study species

Catopsis nitida occurs in Central America from Mexico to Panama, and on islands in the Caribbean (Table 1). It is believed to be pollinated by moths, and has wind-dispersed seeds (Haber, 2000; Morales, 2003; Palací et al., 2004a). This species is easily identified in the field by its vegetative and floral features—narrow and cylindrical rosettes with tubular, long leaves (Morales, 2003). The slender tight rosette of leaves creates a water-loading tank that is characteristic of this genus (Palací et al., 2004b). *Catopsis nitida* grows epiphytically

TABLE 1. Local abundance in each habitat, genetic differentiation (F_{ST}), pollinator, and dispersal syndrome for two bromeliad species.

Global distribution	*Catopsis nitida*	*Werauhia tonduziana*
	Central America, Caribbean islands	Costa Rica
Habitat- local abundance		
Primary forest	0	++
Mixed forest	++	+
Pasture	++	++
Genetic differentiation		
Among habitats	0.26	0.13
Among pastures	0.23	0.15
Isolation by distance	no	weak
Pollinator and dispersal syndrome		
Pollinator	small moth	hawkmoth/bat
Dispersal	wind	wind

Notes: ++ represents most abundant, + present but infrequently abundant, and 0 represents absent.
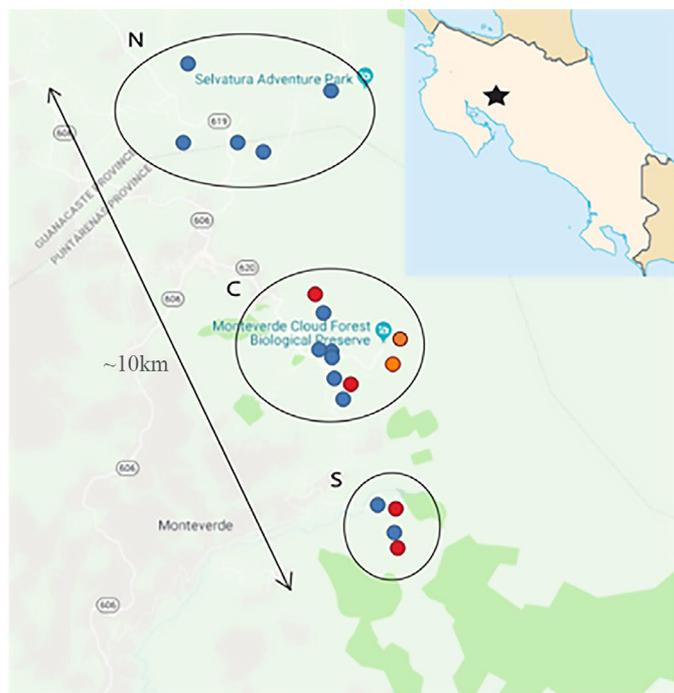
FIGURE 1. At the top right, location of the Monteverde region in Costa Rica (Google Maps). On the left, a close-up of the Monteverde study region. Pasture (blue markers), primary forest (orange) and mixed forest (red) population locations are indicated. Black circles represent northern, central, and southern regional groups designated for genetic structure analyses.

on trees, and often in clonal ramets (Appendix S2). Some authors suggest that this species is dioecious (Utley, 1994; Haber, 2000), but other authors and some herbarium records describe *C. nitida* as perfect-flowered and hermaphroditic (Brown and Gilmartin, 1989; Espejo-Serna et al., 2005). As discussed later, our genetic data suggest that *C. nitida* may frequently self-fertilize.

Werauhia tonduziana grows epiphytically from a rosette composed of dark green leaves, produces nocturnal flowers that are exerted from the underside of the flower bracts, and is endemic to Costa Rica (Morales, 2003). This species is likely hawkmoth- or bat-pollinated and also has wind-dispersed seeds (Haber, 2000; Morales, 2003). *Werauhia tonduziana* is believed to be hermaphroditic (Haber, 2000), and our genetic data support this inference. Voucher specimens for each of the two species in this study are deposited at the National Museum of Costa Rica (CR296386 *Catopsis nitida*, CR296387 *Werauhia tonduziana*).

Laboratory procedures

From each tree, leaf samples were collected from 1–3 mature epiphyte individuals in pastures and 1–11 mature epiphyte individuals in forests. The samples were dried on silica gel in coin envelopes for transport to our laboratory at the University of Utah. From each leaf sample, 10–20 mg of dried material was ground in a Minilys tissue homogenizer (Bertin, Bretonneux, France) with 11–12 ceramic beads, and genomic DNA was extracted using a generic spin-column protocol as described in Appendix S3. We genotyped individuals at eight microsatellite loci using primers developed for this study. Dye-labeled PCR products were resolved by capillary

electrophoresis, and alleles were called using Peak Scanner Software (Version 2.0, Thermo Fisher Scientific). Seven of the eight microsatellite loci developed for *Catopsis nitida* are tri- or tetra-nucleotide repeats, which tend to have lower mutation rates and therefore lower levels of standing polymorphism (other things equal) than do loci consisting of dinucleotide repeats (one locus in *C. nitida*, and all eight loci in *Werauhia tonduziana*). Aspects of microsatellite primer development, PCR, genotyping, and facts about the loci including their overall allelic diversities are described in Appendix S3.

Local abundance

We assessed the presence or absence of both species in the primary forest of the Monteverde Cloud Forest Reserve and four mixed forest sites (Crandall Reserve, and three other private properties, Costa Rica). We used arborist climbing techniques to gain access to canopy dominant trees in these sites (Perry, 1978; Anderson et al., 2015). We selected trees that were 100–200 m apart from each other, while attempting to maximize tree species diversity and climbing safety. In the crown of each tree, we counted the number of individuals of each bromeliad species on 8–12 branches. We also observed five to ten other trees surrounding the focal tree and counted the numbers of visible individuals of each of the two bromeliad species, using binoculars. Because primary and mixed forest trees often required climbing for access to epiphytes, we typically did not score as many trees as in pastures where trees are often shorter and visually accessible from the ground. To compare our findings of local abundance to global records, we reviewed all electronic of the herbarium records with information regarding habitat type for *Catopsis nitida* and *Werauhia tonduziana* from the online database *Tropicos* (https://www.tropicos.org/).

Estimates of genetic diversity and self-fertilization

For both species, the numbers and identities of the alleles present per locus in each population, their frequencies, and various measures of genotypic diversity were estimated using the R packages adegenet and diveRsity (Keenan et al., 2013). FreeNA (Chapuis and Estoup, 2007) and INEST 2.2 (Chybicki and Burczyk, 2009) were used to estimate null-allele frequencies together with subpopulation differentiation (F_{ST}) or inbreeding coefficients (F_{IS}, F_{IT}) corrected for the presence of such non-amplifying "nulls". Being invisible, nulls can inflate estimates of homozygosity. We used INEST's null-corrected allele-frequency estimates to derive expected heterozygosity levels, which provide a natural measure of genetic diversity. We estimated upper limits on the selfing rate as $S = 2F_{IS}/(1+F_{IS})$ (Pollak, 1987; Chauvet et al., 2004). This equation assumes that the population is in equilibrium, and that self-fertilization is the only process contributing to excess homozygosity at the subpopulation level. Biparental inbreeding (where there are two parents, often closely related) can also contribute to the excess homozygosity that is used to estimate F_{IS} and thereby S, but self-fertilization will tend to have a larger effect on homozygosity if it takes place frequently and migration rates are not extremely low.

Estimated null-allele frequencies were consistently low (median $p = 0.020$ and 0.027 in *Catopsis nitida* and *Werauhia tonduziana*, respectively); their estimation only slightly reduced estimates of inbreeding and population differentiation (Appendix S4); and when we compared INEST model fits with and without the estimation of null-allele frequencies, the DIC (deviance information criterion)

statistics were ambiguous as to whether the models that include nulls were preferred.

Genetic differentiation and structure analyses

For each species, we ran three analyses of molecular variation (AMOVA) in Arlequin 3.5.2.2 (Excoffier and Lischer, 2010). First, we designated primary forest, mixed forest, and pasture habitats as the highest grouping level, with populations representing separate forest and pasture sampling sites. Second, we considered the pasture populations separately as a single, otherwise unstructured grouping. And third, we considered the pasture populations grouped into northern, central, and southern regions (Fig. 1). We compared FreeNA's null-allele corrected pairwise F_{ST} estimates to F_{ST} estimates calculated without correction for nulls in Arlequin (Chapuis and Estoup, 2007). We examined isolation by distance within species by regressing pairwise differentiation (F_{ST}) on the average distance between populations using Mantel's test. We calculated genetic distances (F_{ST}) among populations and both geographic and Euclidean physical distances using Arlequin 3.5.2.2 and GenoDive software (Meirmans and Van Tienderen, 2004; Excoffier and Lischer, 2010) and performed the Mantel's test in RStudio Version 1.1.447 (RStudio Team, 2015) using the ecodist package (Goslee and Urban, 2007). We also quantified the signal of isolation by distance for each species by regressing FST 1 ($F_{ST}/1-F_{ST}$) on log geographic distance (Rousette, 1997).

RESULTS

Presence of the species in different habitat types

Catopsis nitida individuals were most prevalent on trees in pastures and mixed forest (Table 1). We found no *C. nitida* in our primary forest sites (Table 1). *Werauhia tonduziana* were most prevalent on primary forest and pasture trees, but were also found on mixed forest trees (Table 1), consistent with global records for the two species. There were 63 herbarium records with information regarding habitat type for *C. nitida* in the online database *Tropicos*. Records for *C. nitida* came from the Dominican Republic, Puerto Rico, Costa Rica, Nicaragua, and Panama. For many records, the forest type included altered habitats (24 records, or 38%). These altered habitats included disturbed or secondary forest, pastures or agricultural areas, pasture/forest interface or pasture/forest edge, gaps, and along roadways. There were records from forest habitats but only two of these records include the term "primary forest". *Werauhia tonduziana* is endemic to Costa Rica and has few herbarium records in the *Tropicos* database, so we have little information on its preferred forest types, apart from our own data.

Levels and patterns of genetic diversity in *Catopsis nitida*

Allele numbers and heterozygosity levels were generally low in *Catopsis nitida*, as expected given that all but one of its microsatellite loci are tri- and tetra-nucleotide repeats (Table 2 and Appendix S3b). The one dinucleotide repeat locus had 8 alleles and vastly higher heterozygosity levels, approaching those seen in *Werauhia tonduziana* (Table 2 and Appendix S3b). At all loci, observed heterozygosity levels were generally lower than the levels expected under random mating, and the size of this effect (the heterozygote deficit or homozygote excess) increased with the size of the demographic unit being evaluated (tree, population, region or the entire study) (Table 2, Appendices S4a and S4b), as is expected for a geographically structured population with limited gene flow. As a consequence, individual inbreeding coefficients (F_{IS} or F_{IT}) also increased with the size of the reference population, being much lower for the individuals on a tree than for those on all the trees in a population, and so on.

If individuals either self-fertilize (with probability S), or outcross randomly with a member of their local subpopulation (1-S), then the selfing rate can be estimated as $S = 2F_{IS}/(1+F_{IS})$. For six trees from which we sampled three or more *Catopsis nitida* individuals, the average null-corrected within-tree $F_{IS} \approx$ 0.2 (Appendix S4a), implying $S \approx 0.33$. This estimate should be viewed as a rough upper limit on the range of plausible selfing rates, because biparental mating between close relatives will also contribute to F_{IS}. However, high rates of familial inbreeding would be required to maintain an average F_{IS} as high as 0.2 without any contribution from selfing, and it is not clear how such rates could be sustained in an insect-pollinated, wind-dispersed plant. Thus, the true selfing rate for *C. nitida* seems likely to fall in the range $S \approx 0.2$ to 0.3.

Levels and patterns of genetic diversity in *Werauhia tonduziana*

Allele numbers and heterozygosities were much higher in *W. tonduziana*, as expected given that its microsatellite loci are all dinucleotide repeats which tend to have high mutation rates (Tables 2 and Appendix S3b). However, just as in *Catopsis nitida*, homozygote excesses (heterozygote deficits) were ubiquitous, and they increased with the level of population aggregation as expected for a geographically structured species. And as in *C. nitida*, F_{IS} values at the lowest level of aggregation (plants on the same tree) imply a mating system of predominant outcrossing with modest but significant rates of self-fertilization. For 10 trees with three to 11 sampled individuals, the average $F_{IS} \approx 0.14$, implying a theoretical selfing rate of $S \approx$ 0.24, which as argued above, would seem likely to be at least 0.15 in reality (Appendix S4a).

Genetic structure of *Catopsis nitida*

Overall, there was significant genetic differentiation among populations with 26% of the variation distributed among populations ($F_{ST} = 0.26$, $P < 0.001$, Table 3). Within habitat types (pasture or mixed forest), populations were also differentiated ($F_{SC} = 0.24$, $P < 0.001$, Table 3). However, there was no significant differentiation between the pasture and mixed forest groups as a whole ($F_{CT} = 0.03$, $P = 0.19$, Table 3). Pasture populations considered on their own varied considerably among themselves ($F_{ST} = 0.22$, $P < 0.001$). When we grouped the pasture populations regionally into northern, central, and southern groups, we also found significant differentiation among the local populations within their regional groups ($F_{ST} = 0.23$, $P < 0.001$, Table 3), and among these populations within the regional groups ($F_{SC} = 0.20$, $P < 0.001$, Table 3). However, the variance among regional groups relative to the total variance was not significant ($F_{CT} = 0.03$, $P = 0.12$, Table 3). Although slightly lower on average, null-allele corrected F_{ST} values were similar to global F_{ST} values without such correction (Appendix S4b).

TABLE 2. Summary of genetic variation among forest and pasture populations of *Catopsis nitida* and *Werauhia tonduziana* with number of individual epiphytes sampled (*N*), number of trees from which epiphytes were sampled (*Nt*), observed heterozygosity (*Hobs*), expected heterozygosity (*Hexp*), expected heterozygosity corrected by estimated null-allele frequencies (*Hexp_null*), and null-corrected inbreeding coefficients (F_{IS}_null). *Hobs* and *Hexp* were estimated using diveRsity (Keenan et al., 2013) and *Hexp_null* and F_{IS}_null were estimated using INEST 2.2 (Chybicki and Burczyk, 2009).

Catopsis nitida							
Habitat Type	Population	*N*	*Nt*	*Hobs*	*Hexp*	*Hexp_null*	F_{IS}_null
Mixed Forest	1	14	3	0.07	0.14	0.17	0.37
	2	14	12	0.17	0.25	0.30	0.26
	3	32	15	0.11	0.17	0.19	0.33
Pasture	1	21	20	0.11	0.20	0.24	0.40
	2	25	25	0.14	0.24	0.29	0.25
	3	19	19	0.13	0.19	0.27	0.16
	4	16	16	0.06	0.23	0.25	0.68
	5	12	12	0.07	0.18	0.23	0.41
	6	2	2	0.12	0.08	0.09	0.11
	7	27	27	0.11	0.21	0.26	0.46
	8	27	25	0.12	0.28	0.30	0.57
	9	8	8	0.09	0.22	0.28	0.54
	10	4	4	0.12	0.09	0.12	0.12
	11	31	29	0.09	0.29	0.33	0.67
	12	7	7	0.23	0.26	0.30	0.09
	13	3	3	0.04	0.20	0.23	0.60
	14	3	2	0.00	0.19	0.22	0.88
Werauhia tonduziana							
Habitat Type	Population	*N*	*Nt*	*Hobs*	*Hexp*	*Hexp_null*	F_{IS}_null
Primary Forest	1	18	8	0.48	0.70	0.81	0.58
	2	25	10	0.32	0.73	0.80	0.17
Mixed Forest	3	2	2	0.38	0.38	0.45	0.09
	4	29	6	0.33	0.48	0.56	0.28
Pasture	2	14	10	0.37	0.55	0.63	0.31
	3	45	45	0.37	0.60	0.70	0.33
	6	6	6	0.36	0.52	0.65	0.19
	7	7	7	0.45	0.58	0.67	0.13
	8	36	30	0.47	0.64	0.71	0.07
	9	8	8	0.3	0.57	0.63	0.44
	10	6	6	0.48	0.6	0.72	0.16
	11	28	28	0.32	0.56	0.68	0.36
	12	18	16	0.24	0.66	0.73	0.63
	13	32	26	0.35	0.58	0.64	0.37

Although F_{ST} generally tends to increase with distance, the Mantel's test did not indicate significant relationships between genetic differentiation (F_{ST}) and geographic or Euclidean distances between populations ($r = 0.189$, $P = 0.115$ and $r = 0.167$, $P = 0.170$, respectively). The regression of FST 1 on log geographic distance indicated a weak but significantly positive relationship ($P = 0.019$). However, the distribution of pairwise F_{ST} between populations shows considerable scatter that is not geographically structured (Fig. 2A). Populations that are closer to one another or within the same regional grouping (northern, central, southern) were not necessarily more genetically similar. Many of the highest differentiation scores ($F_{ST} > 0.5$) are those between mixed forest and pasture populations (Fig. 2A).

Genetic structure of *Werauhia tonduziana*

Populations of *W. tonduziana* were significantly differentiated overall ($F_{ST} = 0.13$, $P < 0.001$, Table 3), but only half as strongly as populations of *Catopsis nitida*. Within the primary forest, mixed forest, and pasture groups, populations differed substantially in a hierarchical analysis ($F_{SC} = 0.13$, $P < 0.001$), as did the pastures considered separately ($F_{ST} = 0.15$, $P < 0.001$, Table 3). When the pasture populations are grouped regionally into northern, central, and southern groups, given the amount of total population differentiation ($F_{ST} = 0.15$), the majority (80%) of this F_{ST} value is accounted for by differences among groups ($F_{CT} = 0.12$, $P < 0.001$) and little (20%) is accounted for by differences among pastures within regional groups ($F_{SC} = 0.03$, $P = 0.003$, Table 3), although that component remains significant. Although slightly lower on average, null-corrected F_{ST} values were similar to uncorrected values (Appendix S4b).

Pairwise F_{ST} increased with geographic distance in *Werauhia tonduziana*. Although not quite formally significant, the Mantel's test indicates that genetic relatedness declines with physical distance ($r = 0.284$, $P = 0.060$), and the regression of FST 1 on log geographic distance is significantly positive ($P = 0.007$). Populations closer to one another or within the same regional grouping (northern, central, southern) were more genetically similar, and populations in the southern group were notably distinct (Fig. 2B). Primary forest population 2 was genetically distinct from all other populations, whereas primary forest population 1 was relatively similar to most other populations across the region (Fig. 2B).

TABLE 3. Analysis of molecular variance results for *Catopsis nitida* and *Werauhia tonduziana* calculated using Arlequin.

Source of variance- level of analysis	*df*	Sum of squares	Variance components	Percent of variation	*F*-Statistic	*P*-value
Catopsis nitida- mixed forest:pasture comparison						
Among habitats	1	20.85	0.04	2.97	$F_{CT} = 0.03$	0.19
Among populations within mixed forest or pasture habitat	15	138.63	0.29	23.3	$F_{SC} = 0.24$, $F_{ST} = 0.26$	< 0.001, < 0.001
Within populations	507-513	461.13	0.90	73.7		
C. nitida- pasture only						
Among geographic groups (northern, central, southern)	2	35.82	0.04	3.48	$F_{CT} = 0.03$	0.12
Among pasture populations within geographic groups	11	75.8	0.24	19.66	$F_{SC} = 0.20$, $F_{ST} = 0.23$	< 0.001, < 0.001
Within pasture populations	390-396	373.38	0.95	76.9		
Werauhia tonduziana- primary forest:mixed forest:pasture comparison						
Among habitats	2	39.08	−0.01	−0.22	$F_{CT} = -0.002$	0.48
Among populations within primary forest, mixed forest, or pasture habitat	11	165.51	0.36	12.68	$F_{SC} = 0.13$, $F_{ST} = 0.13$	< 0.001, < 0.001
Within populations	498-534	1303	2.46	87.54		
W. tonduziana- pasture only						
Among geographic groups (northern, central, southern)	2	98.76	0.36	12.44	$F_{CT} = 0.12$	< 0.001
Among pasture populations within geographic groups	7	33.57	0.07	2.57	$F_{SC} = 0.03$, $F_{ST} = 0.15$	0.003, < 0.001
Within pasture populations	358-390	942.72	2.44	84.98		

DISCUSSION

Our main aim was to investigate the population-genetic fates of epiphyte populations on isolated pasture trees, and their possible roles in the maintenance of genetic connectivity over increasingly fragmented tropical forest landscapes. Both study species (*Catopsis nitida* and *Werauhia tonduziana*) show genetic structuring, indicating low average rates of pollen and seed migration over scales of a few kilometers. These patterns differ quantitatively in interesting ways that may reflect the behaviors of their pollinators. Both species also appear to self-pollinate at low but meaningful rates (probably 15 to 25% of seeds). These factors would seem to suggest that small populations on isolated pasture trees might be highly vulnerable to loss of genetic diversity via drift. But in fact, those populations are almost as diverse, on average, as their conspecific forest populations. This pattern implies that pasture trees receive new immigrating alleles at rates that tend to offset their losses from drift, and that, to the degree they also send emigrating alleles to other pasture trees and neighboring forests (which is implied by symmetry), they also contribute to the regional connectivity of their species.

Genetic differentiation among populations across the Monteverde region was far greater for *Catopsis nitida* than for *Werauhia tonduziana*. This difference could suggest that *W. tonduziana* populations may have higher genetic connectivity compared to *C. nitida* populations across this fragmented landscape. Unlike *W. tonduziana*, *C. nitida* showed substantial differentiation among almost all populations, regardless of distance, and did not show a clear pattern among regions.

Gene flow and connectivity in plants may be affected by the mating system, pollen movement, seed dispersal, and colonization and establishment success (Allendorf et al., 2013). The differences in population structuring and genetic differentiation we documented suggest that factors affecting gene flow have different effects on these two species across this fragmented landscape. Both species show moderate to high levels of inbreeding within populations (F_{IS}), moderate to high levels of total population inbreeding (F_{IT}), and moderate self-fertilization rates. Selfing and other forms of inbreeding can reduce the effective population size and decrease allelic diversity within populations. As a result, selfing species typically have higher F_{ST} values; however, high rates of selfing and excess homozygosity within populations cannot alone explain the population-level patterns of differentiation that we found in these two species. Similarly, in a study examining patterns of differentiation in the bromeliad *Guzmania monostachia* (L.) Rusby ex Mez in fragmented forests of Costa Rica, Cascante-Marín et al. (2014) found that gene flow among forest fragments was affected by this species' autogamous mating system and also by its dispersal mechanisms. For *G. monostachia*, genetic relatedness was high within forest patches, but there was little differentiation among patches because gene flow (mediated mainly by dispersal) was high enough to offset the differentiating effects of genetic drift within patches.

The distinct patterns of genetic structuring in the two bromeliad species studied here may reflect their dispersal syndromes and the behaviors of their pollinators. In *Catopsis nitida*, neighboring populations do not tend to be closely related, and distant populations are not necessarily more genetically distinct, as has been reported for another species of *Catopsis*, which exhibits episodic long-distance dispersal (Cascante-Marín et al., 2006a, b, 2009, 2014). This suggests that although mating among near neighbors is frequent within populations, infrequent pollen movement by moths and/or long-distance wind-dispersal of seeds may allow for limited, episodic gene flow among populations. About 85% of the F_{ST} value was found among pastures within regions, which suggests that moths may be transporting pollen locally, but seldom over long distances, and that seeds may occasionally experience long-distance dispersal and establishment. Where pollinators do not move great distances and exhibit "trap-lining" behavior (from flower to nearby flower), mating between plants may fall off rapidly with distance

A

Northern

	Pasture 12	Pasture 13	Pasture 7	Pasture 8	Pasture 6	Mixed Forest 2	Pasture 4	Pasture 1	Pasture 10	Pasture 9	Pasture 2	Pasture 3	Pasture 5	Pasture 14	Mixed forest 1	Pasture 11
Pasture 12																
Pasture 13	0.302															
Pasture 7	0.345	0.411														
Pasture 8	0.16	0.16	0.082													
Pasture 6	0.234	0.172	0.391	0.09												
Mixed Forest 2	0.123	0.119	0.288	0.13	0.27											
Pasture 4	0.241	0.308	0.219	0.163	0.397	0.085										
Pasture 1	0.302	0.246	0.265	0.132	0.292	0.157	0.299									
Pasture 10	0.154	0.506	0.546	0.387	0.684	0.242	0.345	0.458								
Pasture 9	0.196	0.188	0.282	0.13	0.303	0.033	0.176	0.031	0.439							
Pasture 2	0.087	0.341	0.342	0.238	0.372	0.187	0.212	0.324	0.083	0.214						
Pasture 3	0.056	0.391	0.433	0.291	0.443	0.085	0.295	0.408	0.088	0.308	0.02					
Pasture 5	0.077	0.374	0.406	0.253	0.452	0.04	0.268	0.344	0.087	0.235	-0.003	-0.01				
Pasture 14	-0.032	0.223	0.415	0.169	0.154	0.196	0.352	0.313	0.311	0.243	0.175	0.236	0.2			
Mixed forest 1	0.308	0.637	0.43	0.389	0.663	0.425	0.323	0.586	0.451	0.525	0.238	0.184	0.364	0.473		
Pasture 11	0.022	0.163	0.267	0.115	0.161	0.064	0.132	0.197	0.126	0.1	0.076	0.097	0.071	0.024	0.273	
Mixed forest 3	0.129	0.449	0.5	0.372	0.522	0.243	0.313	0.439	-0.034	0.316	0.062	0.04	0.038	0.247	0.336	0.118

Row groupings: Northern (Pasture 12, Pasture 13, Pasture 7, Pasture 8, Pasture 6); Central (Mixed Forest 2, Pasture 4, Pasture 1, Pasture 10, Pasture 9, Pasture 2, Pasture 3, Pasture 5); Southern (Pasture 14, Mixed forest 1, Pasture 11, Mixed forest 3). Column groupings: Northern, Central, Southern.

B

	Pasture 12	Pasture 13	Pasture 7	Pasture 8	Pasture 6	Pasture 10	Pasture 9	Pasture 2	Mixed Forest 4	Pasture 3	Primary Forest 1	Primary Forest 2	Pasture 11
Pasture 12													
Pasture 13	0.02												
Pasture 7	0.03	0.015											
Pasture 8	0.033	0.019	0.04										
Pasture 6	0.022	0.004	0.052	0.061									
Pasture 10	0.02	0.02	0.041	0.014	0.06								
Pasture 9	0.031	0.043	0.077	0.05	0.041	0.034							
Pasture 2	0.035	0.009	0.056	0.01	0.059	0.006	0.047						
Mixed Forest 4	0.066	0.046	0.081	0.035	0.111	0.01	0.079	0.021					
Pasture 3	0.032	0.028	0.057	0.016	0.072	0.007	0.042	0.007	0.045				
Primary Forest 1	0.013	0.024	0.04	0.014	0.048	-0.006	0.021	0.021	0.047	0.016			
Primary Forest 2	0.122	0.187	0.178	0.158	0.193	0.136	0.164	0.183	0.228	0.16	0.106		
Pasture 11	0.26	0.31	0.3	0.31	0.316	0.316	0.218	0.337	0.377	0.29	0.27	0.291	
Mixed Forest 3	0.266	0.34	0.314	0.338	0.365	0.345	0.24	0.385	0.44	0.326	0.289	0.298	-0.02

Row groupings: Northern (Pasture 12, Pasture 13, Pasture 7, Pasture 8, Pasture 6); Central (Pasture 10, Pasture 9, Pasture 2, Mixed Forest 4, Pasture 3, Primary Forest 1, Primary Forest 2); Southern (Pasture 11, Mixed Forest 3). Column groupings: Northern, Central, Southern.

Pairwise F_{ST} legend: <0.05 | 0.05-0.1 | 0.11-0.2 | 0.21-0.3 | 0.31-0.5 | 0.51+

FIGURE 2. (A) *Catopsis nitida*- Mixed forest and pasture population pairwise F_{ST} comparisons, and (B) *Werauhia tonduziana*- Primary forest, mixed forest, and pasture population pairwise F_{ST} comparisons.

(Gaino et al., 2010). This potential explanation for the different genetic structures of these two epiphyte species could be tested by a study that positively identified their pollinators, and the behaviors of those pollinators.

Gene flow in *Werauhia tonduziana* appears to follow the isolation by distance model much more closely than in *Catopsis nitida*, consistent with the hypothesis that *W. tonduziana* experiences lower and less structured barriers to gene flow (Finlay et al., 2017). For pasture populations of *W. tonduziana,* about 80% of the F_{ST} value was contributed by differences between regions, indicating much more regional gene flow as compared to *C. nitida*. Pollinators with larger territories and foraging distances, such as bats and hawkmoths (the currently inferred potential pollinators of this species), may disperse pollen over larger distances, which may offset the effects of fragmentation and isolation (Bacles et al., 2006). Two bat-pollinated bromeliad species, *Alcantarea glaziouana* (Lemaire) Leme and *A. regina* (Vellozo) Harms, had 17 alleles and up to 10

alleles per locus, respectively, for dinucleotide markers (Barbará et al., 2009). Similar to our findings, that study found no significant correlation in the Mantel's test comparing genetic and geographic distances. These results, along with Bayesian analyses, suggested the possibility of occasional long-distance gene flow among island inselbergs for these species because of wide-ranging bat pollination. Bats and hawkmoths have the potential to move pollen great distances, which can greatly slow the rate of differentiation (Finger et al., 2014). Although bats have been found to be hesitant to move into open and fragmented habitats, when there were attractive food rewards in isolated remnant trees, birds and bats made use of those pasture habitats (Werner et al., 2005).

We frequently found *Catopsis nitida* in pasture trees and in mixed forests, but never in primary forest sites, consistent with the historical herbarium records. A previous study in the lower montane cloud forests of Mexico found *C. nitida* inhabiting riparian-forested areas at a rate of 26.8 to 35.5%, while occurring in

slightly lower frequency on isolated pasture trees at 19.1% (Flores-Palacios and García-Franco, 2008). Similar to our study, in the premontane forests of Costa Rica there was a low abundance of *C. nitida* in mature forests, averaging just one individual per 300 m² (Cascante-Marín et al., 2009). This difference may be an additional factor affecting their patterns of genetic variation, as suggested by the propagule-pool model that can increase F_{ST} values among populations (Slatkin, 1977). These findings suggest that *C. nitida* may have colonized isolated trees and fragmented forest sites from a long-distance source population, relatively recently, and that it will remain largely restricted to these habitats. Conversely, *Werauhia tonduziana* showed lower levels of differentiation and was found in all three habitat types. Its weaker population structuring indicates stronger genetic connectivity and suggests that it has probably been in the area for a long time. In addition, pasture trees may be enhancing its gene flow.

CONCLUSIONS

Spatial isolation of trees in pastures can promote increased genetic differentiation among epiphyte populations in pastures as compared to those in adjacent forests. Because of the moderate degrees of differentiation in both species, genetic conservation of these two epiphytic species would require representation of individuals and genotypes across the region, and include individuals from both forest and pasture habitats. Though genetic connectivity may currently be weaker than it was in the formerly intact forests, pasture trees and mixed forest fragments allow some species to remain relatively well-connected despite fragmentation (Boshier, 2004; Prevedello et al., 2018). Therefore, isolated pasture trees are potentially important habitats for epiphytes, providing both "landing" and "launching" sites for colonization, and potentially promoting gene flow among the larger populations that occur in forest patches.

Although trees in pastures cannot offset the loss of intact primary forests (Gibson et al., 2011), maintaining scattered trees for epiphyte habitat should be a goal of future conservation efforts (Lumsden and Bennett, 2005; Manning et al., 2006; Fischer et al., 2010; Lander et al., 2010; Lindenmayer and Laurance, 2016; Prevedello et al., 2018), and where possible, native tree species should be planted because they are more likely to increase gene flow among populations of native epiphytes (Prevedello et al., 2018). Because of their potential importance as mediators of gene flow and dispersal for a major component of tropical plant diversity, pasture epiphytes should also be monitored to broaden and deepen our understanding of their requirements and their roles in maintaining the genetic connectivity of their species in the face of continued forest fragmentation and climate change.

AUTHOR CONTRIBUTIONS

AAA and NMN developed the project. AAA collected the data and conducted the field work. AAA and JS conducted the laboratory work. AAA, JS, and ED conducted data analyses and interpretation. All authors assisted with writing and editing the manuscript.

DATA AVAILABILITY

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.2280gb5n0 (Amici et al., 2019).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Locality and GPS coordinates for each population sampled.

APPENDIX S2. Clonality in *Catopsis nitida*.

APPENDIX S3. DNA extraction, PCR protocol, and number of alleles for each species.

APPENDIX S4. Genetic diversities and heterozygote deficits of epiphyte populations on individual host trees. Global population differentiation statistics (F_{ST}) and average individual inbreeding coefficients (F_{IT}) for each species.

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