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Tropical forest fragmentation limits pollination of a keystone understory herb

Adam S. Hadley,[1,4] Sarah J. K. Frey,[1] W. Douglas Robinson,[2] W. John Kress,[3] and Matthew G. Betts[1]

[1]*Department of Forest Ecosystems and Society, Oregon State University, Corvallis, Oregon 97331 USA*
[2]*Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon 97331 USA*
[3]*Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560 USA*

Abstract. Loss of native vegetation cover is thought to be a major driver of declines in pollination success worldwide. However, it is not well known whether reducing the fragmentation of remaining vegetation can ameliorate these negative effects. We tested the independent effects of composition vs. configuration on the reproductive success of a keystone tropical forest herb (*Heliconia tortuosa*). To do this we designed a large-scale mensurative experiment that independently varied connected forest-patch size (configuration) and surrounding amount of forest (composition). In each patch, we tested whether pollen tubes, fruit, and seed set were associated with these landscape variables. We also captured hummingbirds as an indication of pollinator availability in a subset of patches according to the same design. We found evidence for an effect of configuration on seed set of *H. tortuosa*, but not on other aspects of plant reproduction; proportion of seeds produced increased 40% across the gradient in patch size we observed (0.64 to >1300 ha), independent of the amount of forest in the surrounding landscape at both local and landscape scales. We also found that the availability of pollinators was dependent upon forest configuration; hummingbird capture rates increased three and one-half times across the patch size gradient, independent of forest amount. Finally, pollinator availability was strongly positively correlated with seed set. We hypothesize that the effects of configuration on plant fitness that we observed are due to reduced pollen quality resulting from altered hummingbird availability and/or movement behavior. Our results suggest that prioritizing larger patches of tropical forest may be particularly important for conservation of this species.

Key words: habitat fragmentation; habitat loss; Heliconia; *hummingbird; patch size;* Phaethornis; *pollination; tropical forest.*

Introduction

A major driver of pollination declines is hypothesized to be anthropogenic changes in the amount and distribution of native habitats across landscapes (Aguilar et al. 2006). However, which landscape attributes (e.g., habitat amount, connectivity, patch size) or what particular aspects of the pollination system (e.g., plant demography, pollinator abundance or movement) drive observed pollination failures remain unclear. Composition combines the type and amount of habitat or cover types within a landscape, while configuration reflects the spatial pattern of landscape elements (Turner 1989). We subsequently refer to habitat loss as changes in landscape composition and fragmentation per se as the spatial configuration of remaining habitat, independent of the amount of habitat within the landscape (Fahrig 2003). Separating the effects of habitat loss and fragmentation in ecological research has proven difficult since landscape composition and configuration are

almost always confounded; landscapes with large amounts of habitat are typically unfragmented, while landscapes with low amounts of habitat are usually characterized by small, unconnected patches (Fahrig 2003). To draw inferences about the independent effects of fragmentation, researchers must therefore separate these confounded effects either statistically or through experimental design.

Habitat loss and fragmentation may influence pollination by affecting plant demography, pollinator abundance/diversity, and pollinator movement (Kremen et al. 2007). First, loss and fragmentation of native habitats may reduce plant abundance, density, and health (de Blois et al. 2002), thereby resulting in declines in the total amount of pollen available for transfer. Second, the abundance and diversity of pollinators upon which plants depend may be altered by these landscape changes (Winfree et al. 2011). Finally, fragmentation per se may restrict the movements of pollinators (Tewksbury et al. 2002) or alter their behavior under differing foraging contexts (Chittka and Schurkens 2001), thus reducing their effectiveness in pollen transfer. This reduction in pollen transfer effectiveness in altered landscapes may result from decreases in both pollen quality and quantity

(Aizen and Harder 2007). While landscape changes appear to have widespread negative effects on pollination, the specific processes (e.g., habitat loss or fragmentation) remain elusive (Eckert et al. 2010).

In addition to difficulties in separating effects of composition from those of configuration, landscape-scale pollination studies have proven complicated due to issues relating to scale and low replication. For instance, to reflect the scales that are relevant to pollination, researchers need to examine the spatial extents most likely to be reflective of the movement behavior of pollinators in their system, while also considering changes in plant density at local, patch, and landscape levels (Hadley and Betts 2012).

The potential for landscape change to affect pollination through changes in composition and configuration can be expressed as two hypotheses with differing implications for habitat loss and fragmentation; the landscape composition hypothesis (Fahrig 2013) expects the composition of cover types in the landscape to be important for pollination at scales larger than local plant communities. Under this hypothesis, pollination failure should increase with decreasing amounts of plant and/or pollinator habitat in the surrounding landscape, regardless of configuration. This hypothesis is most likely to be supported if pollination success depends primarily on either the abundance of available flowers or pollinators at broader spatial scales. Landscape composition could also affect pollination via variability in matrix quality (Tscharntke et al. 2012). High quality matrix (i.e., containing alternative flower sources) could allow pollinators to persist in landscapes with low amounts of habitat for a focal plant species by providing options for resource supplementation in the matrix.

The fragmentation hypothesis anticipates that after controlling for effects of habitat loss, pollination success will be influenced by the configuration of the landscape. The effects of fragmentation per se, both negative and positive, can result through edge effects, differences in connectivity, and patch size differences (Fahrig 2003). If either plant or pollinator densities differ, depending on habitat configuration or if pollinator behavior, particularly movement, is influenced by landscape pattern, then pollination success could be affected. Fragmentation is expected to become particularly important when the amount of habitat in the landscape drops below some critical threshold (Andren 1994, Betts et al. 2007).

We test these hypotheses by conducting a large-scale study designed to separate the effects of composition and configuration on pollination and pollinators of a generalist tropical forest herb, *Heliconia tortuosa*. To our knowledge this is the first attempt to disentangle the effects of composition and configuration in a tropical pollination system.

METHODS

The study was conducted in an (~31 000 ha) area of Coto Brus Canton in southern Costa Rica (8°47′7″ N,

82°57′32″ W) surrounding the Organization for Tropical Studies Las Cruces Biological Station. The study region is composed of 43.2% Pacific premontane tropical forest and spans an elevation gradient from ~300 to 1500 m above sea level. The agricultural matrix was previously forested, but is now dominated by pasture (~80% of the matrix) and shade coffee plantations. The majority of land clearing occurred from 1960 to 1990 (Sánchez-Azofeifa et al. 2001), and remaining forest patches span a range of sizes from <1 to >1000 ha across a gradient in forest amount from 1–99% forest within 1 km (Fig. 1).

We selected *Heliconia tortuosa* (Appendix A) as a focal species because it is one of the most common and longest blooming forest-associated ornithophilous plants in our study region. Heliconia species are thought to be keystone mutualists in that they are a bridging species (Dixon 2009, Menz et al. 2011) critical in sustaining a diverse range of large-bodied hummingbird species during periods of food scarcity (Stiles 1975). In addition, a large proportion of the forest hummingbird pollinator community, including important generalist pollinators, visits this species (Borgella et al. 2001), and as such *H. tortuosa* represents an important central node in the ornithophilous plant–pollinator web in this system. This herbaceous species is pollinated primarily by long-billed, traplining hummingbirds, particularly hermits (Stiles and Freeman 1993) and requires pollinator visits for successful sexual reproduction.

Study design

Throughout our study system, changes in composition and configuration are generally highly confounded; if forest patches are randomly sampled within the study area, patch size and amount of forest within 1000 m are highly correlated (Pearson's $r > 0.7$, $P < 0.001$). We used a focal-patch design (Tischendorf and Fahrig 2000, Thornton et al. 2011) to independently vary these two features (See Appendix C for more on focal patch). We used stratified random sampling so that samples represented the full range of variation in forest-patch size (0.64 to >1300 ha) and amount of forested area within a 1000 m radius of the sample site (8–75%; Fig. 1). For the purpose of patch selection we defined two patch size categories (small, <5 ha; large, >40 ha) and two forest amount categories (low, <30% forest; high, >40% forest). We then randomly selected 10 patches within each of these specifications. Patches were selected in ArcMap 9.3 (Esri, Redlands, California, USA) using forest geographic information system (GIS) land cover layers created from a 2-m resolution orthophoto. We chose a 1000 m radius which represents the maximum expected daily movement range of hummingbirds based on experimental translocation data and passive movement observations (Hadley and Betts 2009; Volpe et al., *in press*). Since hummingbirds avoid crossing gaps as small as 30 m (Hadley and Betts 2009), we considered forest patches distinct when they were separated by at
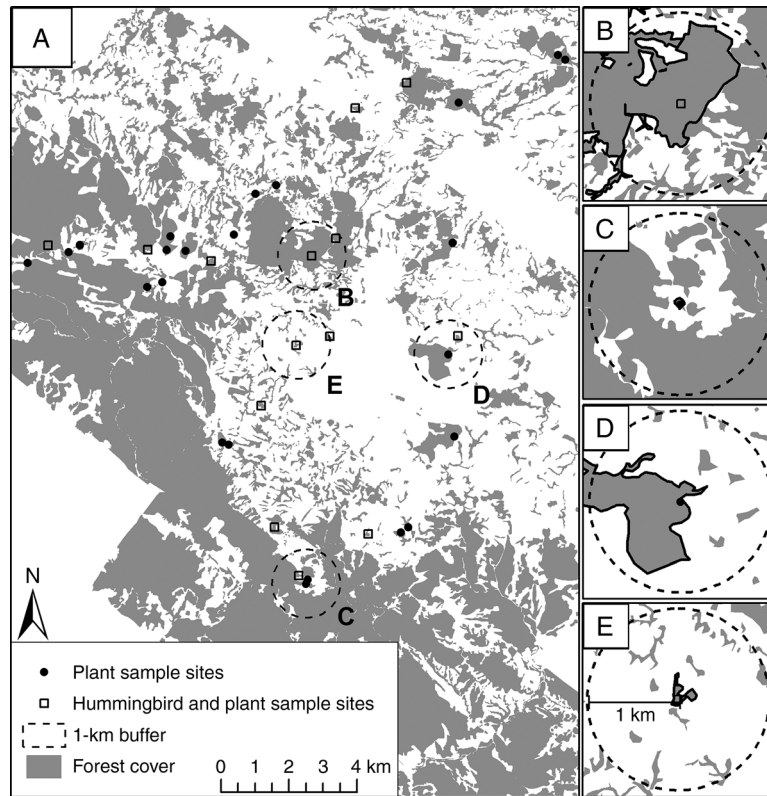
FIG. 1. (A) Map of the study area in Coto Brus, Costa Rica showing the plant and hummingbird sampling locations. Panels B–E are examples of the four broad strata of landscape structure within our stratified random design that enabled us to disentangle configuration (forest-patch size) from composition (forest amount); (B) high percent forest, large patch, (C) high percent forest, small patch, (D) low percent forest, large patch, and (E) low percent forest, small patch. Circles reflect 1000 m radius within which percent forest was calculated.

least 30 m from the nearest forested area. Since forest-patch size and amount of forest could also be positively correlated with elevation due to land-clearing practices in the area, we stratified across two elevation bands (low, 900–1199 m above sea level and high, 1200–1500 m above sea level; 900–1500 m represents the range of *H. tortuosa* within the study area). In 2010, thirty-four suitable patch–landscape combinations were selected according to our stratified random design. In 2011, understory disturbance and restricted patch access prevented us from sampling 11 of the patches sampled in 2010. However, we added three additional randomly selected patches within underrepresented patch-size–forest-amount categories to compensate. Our data set therefore was comprised of 37 forest patches (2010, $n =$ 34; 2011, $n = 26$; Fig. 1). Due to our sampling design, elevation, patch size, and amount of forest were not highly intercorrelated for sites included in our final sample (patch size and amount of forest within 1000 m radius, Pearson's $r = 0.312$, $P = 0.059$; patch size and elevation, $r = 0.013$, $P = 0.43$; and amount of forest and elevation, $r = 0.238$, $P = 0.16$; see Appendix B).

In each forest patch, we sampled plants starting from the patch edge beginning at a randomly selected point

within 500 m from a road access point. From this random point, we selected and marked the first five *H. tortuosa* plants with inflorescences beginning to form. If five flowering *H. tortuosa* were not immediately located, we searched for up to three person-hours longer in an attempt to locate additional plants. In 2010, we sampled 197 plants, and in 2011, 90 new plants were sampled for a combined total of 287 plants across the 37 forest patches.

Composition metrics

To test the landscape composition hypothesis, we examined the influence of three primary variables on various measures of pollination success: (1) the amount of forest in the surrounding landscape, (2) the amount of *H. tortuosa* habitat, and (3) the amount of matrix resources (Appendix B). *H. tortuosa* (Stiles 1975) and its most common pollinators (Borgella et al. 2001) are forest species; however, *H. tortuosa* is patchily distributed within forest, so forest amount is not always correlated with the distribution of this species. For this reason, we modeled *H. tortuosa* distribution using a species distribution model (SDM) and summed this expected distribution at landscape scales (see Appendix

D for detailed methods). To estimate resource quality in the matrix (the area between forest patches) we counted all flower resources visible along roads within our study area. Though roadside surveys clearly have potential biases (Kadmon et al. 2004), this represents a useful assessment of resources available to hummingbirds outside of forest patches; road density is high within the study region (>2 km/km^2) and most of the flowering resources in the matrix were domestic species and located within sight of roads. Nonforested areas far from roads were almost exclusively pastureland and devoid of suitable flowers.

Given some uncertainty in the appropriate scales of investigation, we tested the influence of landscape composition variables (i.e., forest amount, matrix resources) on pollination success at multiple spatial extents. Based on earlier movement work with green hermits (*Phaethornis guy*), we predicted that this hummingbird species spends the majority of time within a relatively small area (<500 m), but also makes longer distance movements (>1000 m) and is familiar with the landscape at larger extents (~2000 m; Hadley and Betts 2009). We therefore investigated the importance of landscape composition metrics within these radii. In addition to these landscape-scale composition metrics, we also examined local-scale forest amount (100 and 250 m radii) to test the importance of composition at smaller spatial scales. This step was important to enable us to discern the independent effects of patch size from the effects of local-scale site composition.

Configuration metric

We derived our measure of configuration based on known behavior of the key hummingbird pollinators in our study area. Our previous results indicate that traplining hummingbirds avoid crossing gaps in forest cover >30 m (Hadley and Betts 2009). Therefore, flower locations that are physically connected to surrounding forest should be more likely to experience greater rates of hummingbird visitation. Since patches are delineated by discontinuities in forest habitat, a plant in a large patch is inherently connected to more forest habitat than plants in small patches. For example, in a large circular patch of 40 ha, pollen can be moved a maximum of 1273 m between plants without pollinators having to cross a nonforested gap. In a small, 1-ha, circular patch, pollen can only be moved a maximum of 64 m before a pollinator is required to cross a gap. Therefore, our metric, area of the patch of forest physically connected to each flower location, was the most ecologically relevant measure of configuration in our system. For brevity, we refer to this metric as patch size. Patch size also has the benefit of being a commonly used metric of configuration in landscape ecology (Hanski and Ovaskainen 2000, Prugh et al. 2008). We used the log of forest-patch size because we expect the biological effects of an equivalent increase in patch size to be greater for a small patch than a large patch (Schmiegelow and

Mönkkönen 2002). We chose not to investigate isolation per se (e.g., Moilanen's connectivity index; Moilanen and Hanski 2001) since most traditional measures of isolation are highly correlated with habitat amount on the landscape and difficult to separate through study design (Bender et al. 2003).

Controlling for nuisance variables

We recorded data on plant size and local environmental features for each plant; this allowed statistical control for possible site-level confounds (Appendix B). Plant vigor is expected to be one of the most important confounds influencing reproductive success (Knight et al. 2005). Unfortunately, lack of ovule fertilization in *H. tortuosa* during our manual pollinations precluded a pollen limitation experiment (A. S. Hadley, *unpublished data*). However, since plant size is thought to be an important indication of *Heliconia* age and vigor (Bruna and Kress 2002), we measured plant height (i.e., from ground to top of the tallest leaf petiole) as well as the number of bracts on the flowering inflorescence. We statistically controlled for plant height in all analyses. We sampled the local flowering community by counting all flowers within 20 m of each focal plant as a measure of conspecific and heterospecific flower densities, both of which have been found to influence plant reproductive success (Kremen et al. 2007). We also quantified distance to the nearest edge, distance to the nearest stream, and the elevation at the plant location, all of which are known to affect growth and distribution of *Heliconia* (Appendix B).

Measures of pollination success

We used three different measures of pollination success, each representing successively higher levels of reproductive information. First, we tested for the presence of pollen tubes; *Heliconia* require pollinator visitation for tubes to be present in styles, but pollen tubes can grow with both self-pollen and outcross pollen (i.e., they are partially self-compatible; Kress 1983). We collected data on pollen tubes by collecting styles from day-old flowers. We inspected the styles for pollen tubes using epifluorescence microscopy (sensu Kress 1983) and recorded the presence or absence of pollen tubes in each style. We examined a style from each of 270 plants distributed across the 37 patches.

We also collected information on fruit and seed set. After the end of flowering but before fruits fully ripened, we covered inflorescences on marked plants with mesh bags. Covering allowed us to wait for all fruits to ripen without fruit being removed by frugivores. We randomly selected two of the bracts on each inflorescence and counted the number of mature fruits out of the total number attempted (the pedicel of unsuccessful flowers can usually still be observed long after the flower has abscised, making an assessment of total flower attempts possible). In *H. tortuosa*, each successful fruit can produce between one and three seeds. We collected all

mature fruits from the two randomly selected bracts on our focal plants and counted the number of seeds contained in each fruit. Similar to the methods for proportion of successful fruits, we controlled for plant reproductive effort by using the proportion of seeds produced out of what was possible given the number of successfully fertilized fruits (i.e., proportion of seeds produced = total number seeds/[total mature fruit $\times$ 3]).

Pollinator availability

To provide data on the pollinator availability, we captured hummingbirds in a subset of forest patches from February to March 2011. We randomly selected 14 forest patches from two groups representing extremes in the patch size and amount of forest gradient (i.e., small forest patches surrounded by low amounts of forest and large forest patches surrounded by a high amount of forest). We captured hummingbirds using 10 mist nets (seven 12-m nets and three 6-m nets) placed in front of ornithophilous flowers within each patch. Area sampled by net arrays was consistent across patch sizes (sampling area vs. patch size correlation, Pearson's $r = -0.250$, $P = 0.39$). Captures were conducted between 05:30 and 12:30 with consistent effort across all patches (17 $\pm$ 0.47 net-hours per patch [mean $\pm$ SD]). Hummingbirds were marked on the head with unique color combinations of nail polish to avoid counting recaptures as separate individuals. Each netting site received three visits separated by a minimum of four days. Order of visits was randomized. We examined the total captures of all hummingbird species considered to be legitimate pollinators of *H. tortuosa* (i.e., those observed to touch the reproductive parts of *Heliconia* flowers when feeding during opportunistic observation periods). These include hermit species (*Phaethornithinae*) and nonhermits with long and/or curved bills that forage in a similar manner to hermits (Stiles and Freeman 1993).

Statistical models

We used linear mixed-effects models (LMEs) to test for the influence of composition, fragmentation, and local nuisance variables on plant fecundity measures. Each forest patch was treated as a random effect and all other variables were treated as fixed effects. Treating individual patches as random effects also removed potential lack of independence caused by repeated sampling of patches ($n = 23$) in both years (Bolker et al. 2009). All models were fit using R 2.15.1 (R Development Core Team 2012) with the lme routine (Bates 2012) using a Gaussian distribution if response variables were continuous and normally distributed (fruit, seed set) or lmer and a binomial distribution (Bates et al. 2012) if the response variable was dichotomous (presence of pollen tubes). We found no evidence of overdispersion but fruit set was zero-inflated (Jackman 2012). Since zero-inflated binomial models (Bolker et al. 2012) failed to converge for the fruit success response variable, we used a two-step approach to account for the zero inflation. First, we examined whether a plant produced at least one successful fruit. Second, within the plants that had at least one successful fruit we looked at the proportion of fruits that were successful. We used Akaike's information criterion corrected for small sample sizes (AIC$_c$) for model selection (Burnham and Anderson 2002).

To limit the total number of models tested, we used the following sequential approach. First, we selected the top nuisance variables. We did this by fitting pollination success models using each local site factor and ranking them using AIC$_c$. If variables in models within two ΔAIC$_c$ points of the top model had parameter estimates whose 90% confidence intervals did not encompass zero, we included them in subsequent models. It is possible that this approach eliminates nuisance variables that might become significant predictors when combined with composition or configuration variables in the final model. However, our use of 90% confidence intervals reduced the possibility of missing important variables. We then selected the top composition variables using the same approach, but controlling for nuisance variables. For example, to test the landscape composition hypothesis we used the top composition and nuisance variables (e.g., pollination $\sim$ nuisance + composition). Once our top local and composition models were determined, we used AIC$_c$ to assess the weight of evidence for the landscape fragmentation hypotheses. We assessed the fragmentation hypothesis by testing for the effect of patch size, statistically controlling for the best variables in the previous two categories (nuisance, composition). Note that despite the fact that our sampling was designed to separate the confounding of composition from configuration, we adopted a more conservative approach of controlling for composition statistically (e.g., pollination $\sim$ nuisance + composition + patch size). If none of the local- (100 m or 250 m radii) or landscape-scale (500 m, 1000 m, or 2000 m radii) composition extents emerged as significant predictors we used the a priori 1000 m radius when controlling for composition. If connected forest-patch size (our configuration metric) is still important after controlling for these variables, then any effects can be attributed to differences in forest configuration associated with patch size per se rather than either local- or landscape-scale effects of forest amount. We tested whether fragmentation effects were amplified at low levels of forest cover by examining the interaction between patch size and composition (e.g., pollination $\sim$ patch size $\times$ composition + local). We analyzed data with both years combined, but also tested whether effects varied interannually (year $\times$ covariate interactions) as well as overdispersion (Jackman 2012). We examined residuals for normality and constant variance. We also used correlograms of Moran's I to test for spatial autocorrelation in Pearson residuals of top landscape model sets. We standardized Moran's I by dividing by its maximum value (after Haining 1990). Because only six

sample sites were within 500 m of each other, our lag intervals were at 700 m to a maximum distance of 18 000 m. We used randomization tests (999 permutations) to determine the probability of observing a value of I as large as the observed value. For each correlogram, the significance of I for each lag distance was calculated using a Bonferroni correction for multiple tests (Legendre and Legendre 1998). This approach is appropriate when autocorrelation is expected at the shortest lags. All of our models contained variables with Pearson's correlations $r < 0.5$ (Appendix B).

RESULTS

Plant fecundity

Pollen tubes were present in 64% of the flowers sampled and widely distributed across 36 of 37 patches. In instances where we detected pollen tubes in a style there were, on average, 2.4 ± 1 tubes per style (mean $\pm$ SD, range = 1–6). Eighty-six percent of the plants produced at least one mature fruit but the average proportion of flowers setting fruit was low (39% $\pm$ 29%, range = 1–100%). Further, within the successfully pollinated fruits only, 46% $\pm$ 20% of possible seeds were produced.

Local environmental variables were effective at predicting plant fecundity, but variable importance depended upon the plant reproduction measure of interest. The presence of pollen tubes was positively associated only with the density of ornithophilous flowers within 20 m of the focal plant (ΔAIC_c to null = 0.55; Appendix E), however this effect was not strong (0.25 ± 0.156, [−0.008, 0.514] [estimate, $\hat{\beta} \pm$ SE, [lower, upper 90% CL]]). Fruit production was also positively associated with the density of ornithophilous flowers ($\hat{\beta} = 0.3 \pm 0.18$, [0.009, 0.600]; Appendix E). Top-ranked local models for successful fruits included plant height and number of bracts on the inflorescence (Appendix E). Both height ($\hat{\beta} = -0.25 \pm 0.087$, [−0.39, −0.104], ΔAIC_c to null = 5.86) and number of bracts ($\hat{\beta} = -0.22 \pm 0.09$, [−0.37, −0.074], ΔAIC_c to top model = 1.86) were negatively associated with the proportion of successful fruits.

Species richness of ornithophilous plants within 20 m was the only local-scale variable found to influence seed production (ΔAIC_c to null = 0.73; Appendix E). Increasing richness of ornithophilous plants showed a weak negative effect on the proportion of possible seeds produced ($\hat{\beta} = -0.029 \pm 0.017$, [−0.001, −0.058]; Fig. 2a). We didn't detect an interaction with year for any of the above effects.

None of our measures of plant reproduction was associated with the amount of forest at local or landscape scales or amount of *Heliconia* habitat (Appendix E); in all cases except proportion of mature fruit the null model received more support. Local-scale forest amount (100 m) was the top model for proportion of mature fruit, but confidence intervals included zero (ΔAIC_c to null = 2.0, $\hat{\beta} = 0.12 \pm 0.1$, [−0.32, 0.17];
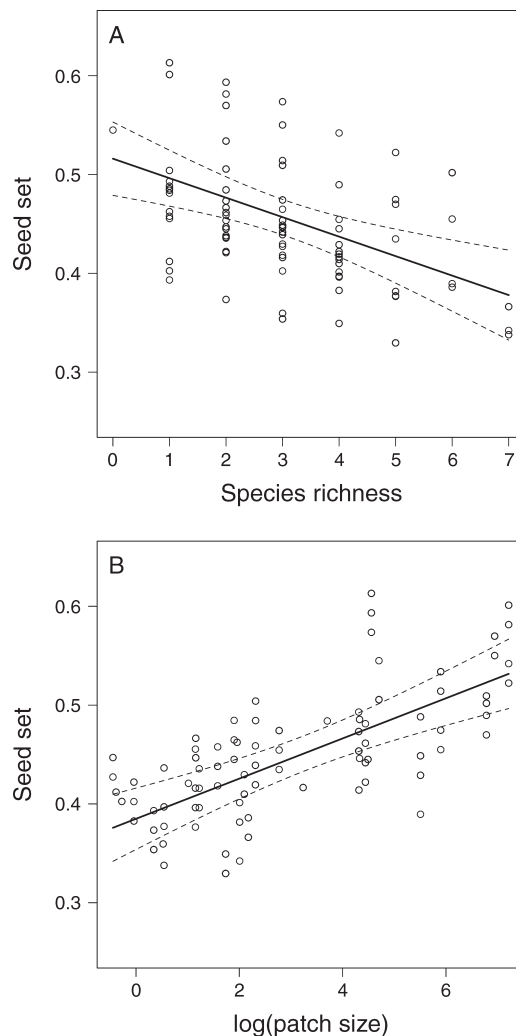

FIG. 2. (A) Increasing species richness of ornithophilous flowers at the local site shows a negative relationship with seed set (measured as proportion of possible seeds that are actually produced; estimate, $\hat{\beta} = -0.03 \pm 0.016$; [−0.057, −0.003] [standardized estimate $\pm$ SE; [lower, upper 90% CL]]). The solid line indicates the modeled relationship between seed set and species richness. The dotted lines represent the standard error of that relationship. The open circles are the fitted values from the model 1 [proportion of possible seeds ~ log patch size + ornithophilous flower species richness, random effect = patch]. (B) Seed set increases with increasing log(forest-patch size) (log [patch size] estimate, $\hat{\beta} = 0.048 \pm 0.018$; [0.017, 0.078]). The solid line indicates the modeled relationship between seed set and log(patch size). The dotted lines represent the standard error of that relationship. The open circles are the fitted values from the model, [proportion of possible seeds ~ log(patch size) + ornithophilous flower species richness, random effect = patch].

Appendix E). Though the amount of resources in the matrix at the 1000-m scale was the top model for explaining the presence of pollen tubes (ΔAIC_c to null = 0.7; Appendix E), this was only a weak positive effect and confidence intervals included zero ($\hat{\beta} = 0.25 \pm 0.16$, [−0.004, 0.519]).

TABLE 1. Comparison of final models testing for effects of configuration, composition, and local variables on the presence of pollen tubes, presence of mature fruit, fruit set, and seed set of *Heliconia tortuosa*.

Model	K	AIC$_c$	ΔAIC$_c$	w_i	ER
Presence of pollen tubes					
Null	2	282.37	0.00	0.36	1.00
pf1000	3	282.81	0.44	0.29	1.25
psize	3	284.42	2.04	0.13	2.78
psize + pf1000	4	284.62	2.25	0.12	3.07
psize × pf1000	5	284.87	2.50	0.10	3.49
Presence of mature fruit					
Flowers	3	231.00	0.00	0.29	1.00
psize + flowers	4	231.16	0.16	0.27	1.08
Null	2	231.92	0.92	0.18	1.58
pf1000 + flowers	4	232.79	1.79	0.12	2.44
psize + pf1000 + flowers	5	233.23	2.23	0.10	3.05
psize × pf1000 + flowers	6	234.96	3.96	0.04	7.25
Proportion mature fruit					
Height + bracts	5	871.83	0.00	0.38	1.00
psize + height + bracts	6	872.32	0.49	0.30	1.28
pf1000 + height + bracts	6	873.45	1.62	0.17	2.24
psize + pf1000 + height + bracts	7	874.41	2.58	0.10	3.63
psize × pf1000 + height + bracts	8	876.54	4.71	0.04	10.54
null	3	877.75	5.92	0.02	19.27
Proportion possible seeds					
psize + richness	5	−19.71	0.00	0.46	1.00
psize × pf1000 + richness	7	−18.40	1.30	0.24	1.92
psize + pf1000 + richness	6	−17.76	1.95	0.17	2.65
Richness	4	−15.73	3.97	0.06	7.29
Null	3	−15.00	4.71	0.04	10.52
pf1000 + richness	5	−13.97	5.74	0.03	17.62

Notes: The number of parameters (*K*), Akaike's information criterion corrected for small sample sizes (AIC$_c$, ΔAIC$_c$), weights (w_i), and evidence ratios (ER) based on the top ranked model are shown. Flowers is total number of ornithophilous flowers, pf1000 is proportion of forest within 1000 m, psize is log(forest-patch size), height is plant height, bracts is number of bracts on the inflorescence, and richness is ornithophilous flower richness.

The presence of pollen tubes was not associated with habitat fragmentation (Table 1). The null model was the best supported and all effects had confidence intervals that included zero (Appendix F). Though the presence of successful fruits and the proportion of mature fruits were best predicted by local factors alone, the second best supported model in both instances included forest-patch size (Appendix F: Table F1b).

There was a 40% increase in seed set across the gradient in patch size we examined. The top model for seed set included a positive effect of forest-patch size ($\hat{\beta}$ = 0.051 ± 0.02, [0.018, 0.084]) even after controlling for local factors and the amount of forest in the surrounding landscape (ΔAIC$_c$ to null = 4.7; Fig. 2b; Appendix F). Patch size was included in the three top-ranked models. The model containing the interaction of patch size and forest amount was the second most supported model (ΔAIC$_c$ to top model = 1.3; Appendix F). The patch size effect was weaker in the second year of the study (year × patch size interaction $\hat{\beta}$ = −0.06 ± 0.034, [−0.12, −0.008]). We detected no spatial autocorrelation in the residuals in our best-supported seed set models (Appendix G). Patch size outperformed competing composition models for seed set at both local- and landscape-scales (local-scale, ΔAIC$_c$ to forest amount within 100 m = 5.27, ΔAIC$_c$ to forest amount within 250 m = 3.8; landscape-scale, ΔAIC$_c$ to top landscape-scale composition metric = 3.50; Appendix H).

Pollinator availability

The capture rate of *H. tortuosa* pollinators (*Phaethornis guy*, *Phaethornis longirostris*, *Campylopterus hemileucurus*, *Heliodoxa jacula*, *Threnetes ruckeri*, and *Glaucis aeneus*; Stiles and Freeman 1993) increased 3.5 times across the gradient in patch size we observed ($\hat{\beta}$ = 6.0 ± 1.4, [2.3, 9.1], R^2 = 0.6, n = 14; Fig. 3a). This positive association of patch size with abundance of pollinators was still present after statistically controlling for the effect of forest amount within the landscape (log patch size $\hat{\beta}$ = 9.23 ± 3.06, [2.5, 16]; proportion of forest $\hat{\beta}$ = −3.63 ± 3, [−10, 3.1], R^2 = 0.65, n = 14). Increasing pollinator availability (log transformed to meet model assumptions) was also strongly associated with mean seed set in capture patches ($\hat{\beta}$ = 0.2 ± 0.07, [0.08, 0.34], R^2 = 0.37, n = 14; Fig. 3b). Seed set increased 37% across the range in pollinator availability that we observed (0.29–1.7 pollinators per net hour).

DISCUSSION

To our knowledge, these results provide the first support for the fragmentation hypothesis in a tropical forest pollination system. Though pollen tubes and fruit set were primarily driven by local factors (e.g., conspecific density and heterospecific plant richness), seed set of *H. tortuosa* increased substantially with the size of tropical forest patches. This effect was independent of both local site conditions and the amount of forest in the landscape, which indicates that forest configuration is important for reproduction of *H. tortuosa*.

To date, relatively few studies have tested for the independent effects of configuration on pollination. Indeed, we know of only two other studies that examine the effects of fragmentation per se on seed set. Diekotter et al. (2007) used a manipulative experiment to examine the effect of patch size on seed set and pollinator visitation rates of red clover (*Trifolium pratense*) in a small-scale experiment, but found no significant differences due to patch size. Contrary to our results, seed set and visitation rates in their small-scale experimental system were driven primarily by total area of clover within treatments and type of matrix, independent of the size of individual clover patches contributing to this area (Diekotter et al. 2007). Farwig et al. (2009) used a mensurative experiment to document a negative effect of decreasing area of connected forest (i.e., patch size) on seed set in a perennial forest species, *Primula elatior*. Similar to our results, they found that seed set was highest for sites physically connected to larger patches of native forest and declined when sites were in smaller forest patches, independent of total amount of forest within a 500 m radius. There are numerous studies documenting apparent patch size effects on fruit or seed set (Aguilar et al. 2006), but because these studies did not separate patch size from habitat loss, we cannot readily interpret how much of the variation is attributable to differences in patch size per se. Nevertheless, our findings suggest that these patch size effects observed in other pollination systems may not be entirely due to the effects of habitat amount (Fahrig 2013).

We found that the capture rate of *Heliconia* pollinators also increased with forest-patch size. This effect was not dependent on the total amount of forest in the landscape and therefore represents an independent effect of forest configuration (Fahrig 2013). Importantly, we also found a strong positive correlation between pollinator availability and seed set. Therefore, reduced reproductive output in *H. tortuosa* in smaller patches could be due to changes in pollinator abundance, changes in pollinator movements (Hadley and Betts 2009), or both.

Despite the positive correlation between pollinator availability and seed set, our observation that pollen tubes were common across all patches supports the hypothesis that increases in seed set as a function of patch size are due to pollinator movement rather than
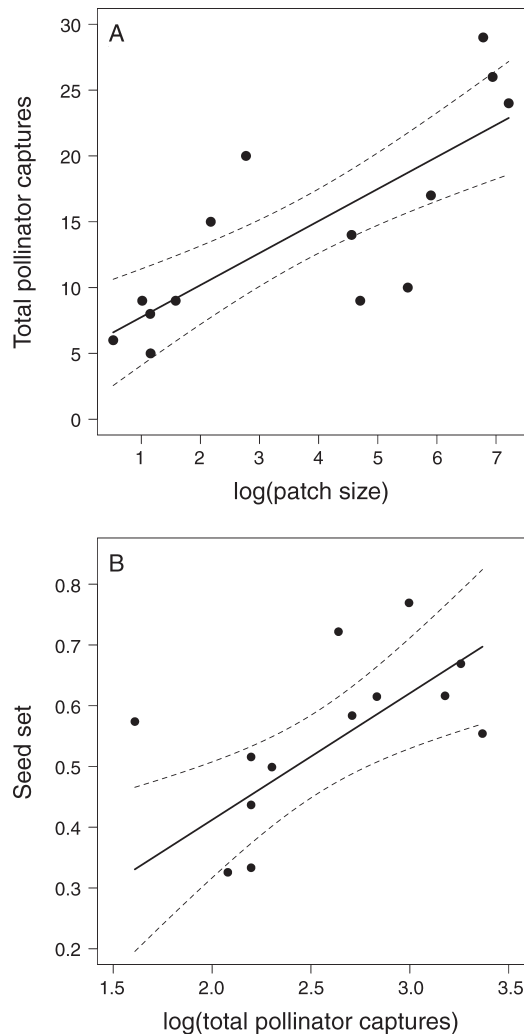

FIG. 3. (A) Number of legitimate *Heliconia tortuosa* pollinators increases with log(forest-patch size) ($\hat{\beta} = 6.0 \pm 1.4$; [2.3, 9.1], $R^2 = 0.6$). The solid line indicates the modeled relationship between pollinator captures and log(patch size). The dotted lines represent the standard error of that relationship. The solid circles are the data points. (B) Increasing pollinator availability shows a positive relationship with seed set ($\hat{\beta} = 0.2 \pm 0.07$; [0.08, 0.34], $R^2 = 0.37$). The solid line indicates the modeled relationship between seed set and log(pollinator captures). The dotted lines represent the standard error of that relationship.

an effect of pollinator abundance alone. As *H. tortuosa* requires pollinator visits to reproduce sexually, the high frequency of pollen tubes in our study shows that the presence of pollinators themselves and resultant pollen quantity is not necessarily limiting. In our study, if pollen tubes were present (79% of plants), there were an average of 2.43 tubes per style. Only 3 tubes per style are required for complete seed set (Kress 1983), so flowers visited by hummingbirds are receiving ~80% of the quantity of pollen required. The percentage of seed set

per fruit is much lower (~45%) so even though a sufficient number of pollen grains germinated, not all ovules were fertilized. This suggests pollen quality limitation (Aizen and Harder 2007), which is often associated with the distance that pollen is transferred.

We propose that fragmentation-induced movement limitation to hummingbirds (Hadley and Betts 2009) results in shorter-distance pollen transfer in small forest patches than within large patches of connected forest. Evidence is accumulating that nonhabitat gaps disrupt the movement of a number of pollinator species in temperate and tropical systems (Townsend and Levey 2005, Hadley and Betts 2009, Cranmer et al. 2012). Therefore, movements across gaps are likely to be less frequent than through connected forest, potentially resulting in lower rates of pollen flow through the matrix. The higher capture rates we observed in large patches could also reflect this reluctance by hummingbirds to regularly cross nonforest gaps resulting in a concentration of resource acquisition to areas of contiguous forest. Together, these observations support the idea that reduced movements by hummingbirds in small patches result in lower quality pollen and subsequent limitation to seed set (Aizen and Harder 2007). An alternative hypothesis is that higher abundance of pollinators in large patches could inflate competition for remaining flower resources thereby potentially increasing specialization (Brosi and Briggs 2013, Fründ et al. 2013) and/or distances covered while foraging; both of these mechanisms are also likely to enhance pollen quality via movement-related mechanisms.

Contrary to our expectations based on the general effects of landscape changes on biodiversity (Fahrig 2003), the amount of tropical forest in the landscape only influenced *H. tortuosa* reproduction when patch size was very small. This is in contrast to studies that have documented substantial effects of forest or habitat amount on pollination (Taki et al. 2007) or pollinators (Holzschuh et al. 2010, Kennedy et al. 2013) in other systems. Further, counter to our expectations, neither the amount of *H. tortuosa* habitat in the landscape or resources available in the matrix appeared to affect pollination.

Not surprisingly, characteristics of the local flowering community were also important correlates of pollination success in *H. tortuosa*, however, effects differed depending on the level of reproductive success examined (i.e., pollen tubes, fruit set, seed set). Presence of both pollen tubes and fruit were positively affected by the total number of ornithophilous flowers within 20 m of the focal plant. Work investigating the effect of floral neighborhood has documented both positive and negative effects of increasing heterospecific densities on pollination (see Knight et al. 2005) with positive effects thought to be due to increased pollinator attraction. In contrast with the effects at lower reproductive levels, seed set was negatively affected by species richness of the local flowering community. This supports the interspe-

cific flower competition hypothesis (Levin and Anderson 1970) that suggests that coflowering species may negatively affect the quality of pollination services by disrupting conspecific outcrossing rates (Fründ et al. 2013). Together, these results suggest that *H. tortuosa* flowers are more likely to be visited when surrounded by many other ornithophilous flowers, but may receive self-pollen or low-quality pollen when higher numbers of heterospecific flowers are present. This effect has been documented in other hummingbird–plant systems (Feinsinger and Tiebout 1991).

The differences in seed set we observed could represent differences in plant vigor for plants in small vs. large forest patches. However, if this were the case, we would expect to find a positive association between forest-patch size and plant size, but we did not find this relationship for either of our plant size measures (height $\hat{\beta} = 0.035 \pm 0.09$, [−0.11, 0.11]; number of bracts $\hat{\beta} = 0.036 \pm 0.08$, [−0.53, 0.17]). Additionally, despite the fact that we would expect plant vigor disparities to arise primarily due to resource differences resulting from increased light availability or moisture, we found no influence of either distance to edge ($\hat{\beta} = −0.02 \pm 0.15$, [−0.05, 0.01]) or streams ($\hat{\beta} = −0.006 \pm 0.02$, [−0.04, 0.02]) on proportional seed production. In all models, we attempted to control for the potential confounding influences of plant vigor (Knight et al. 2005) by including proportional fruit and seed set measures as opposed to absolute fruit and seed production; interestingly, plant size was negatively correlated with the proportion of successful fruits and was not associated with seed set.

In conclusion, our work provides evidence for an independent effect of forest fragmentation on *H. tortuosa* reproduction. Forest-patch size was positively associated with the proportion of seeds produced, independent of forest amount at either local- or landscape-scales. The availability of hummingbird pollinators was also strongly correlated with patch size. In addition, movement patterns of pollinators in this system appear to be restricted by nonforested areas, possibly resulting in higher rates of outcrossing in plants within larger patches where hummingbirds can move further among plants without having to cross gaps. We therefore hypothesize that it is differences in pollen quality resulting from these changes in hummingbird movements and/or availability that are driving the configuration effects on *Heliconia* reproduction we observed. Long-term conservation of this plant species will be best accomplished by maintaining large patches of tropical forest. Establishing connections between existing forest patches could increase connected forest-patch sizes, thereby facilitating movement of pollinators and consequently pollen flow.

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Supplemental Material

Appendix A

Supplementary photo of the study species and additional distribution information (*Ecological Archives* E095-195-A1).

Appendix B

Supplementary table with complete predictor variable correlation matrix and additional information on nuisance variables (*Ecological Archives* E095-195-A2).

Appendix C

Supplementary information on the importance of focal patch design (*Ecological Archives* E095-195-A3).

Appendix D

Supplementary information containing detailed *Heliconia tortuosa* habitat model methods (*Ecological Archives* E095-195-A4).

Appendix E

Supplementary table with preselection of nuisance and landscape composition models for pollination of *H. tortuosa* (*Ecological Archives* E095-195-A5).

Appendix F

Supplementary table showing model averaged estimates for response variables (*Ecological Archives* E095-195-A6).

Appendix G

Supplementary information on testing for any effects of partially overlapping landscapes (*Ecological Archives* E095-195-A7).

Appendix H

Supplementary information on testing competing configuration, local-scale composition, and landscape-scale composition hypotheses (*Ecological Archives* E095-195-A8).