

BIOTIC, GENETIC, AND SPATIAL MECHANISMS CONTRIBUTING TO PROGENY  
SURVIVAL AND NEGATIVE DENSITY DEPENDENCE IN NEOTROPICAL PALM  
SPECIES.

A DISSERTATION ABSTRACT

SUBMITTED ON THE THIRTY FIRST DAY OF AUGUST 2023

TO THE DEPARTMENT OF ECOLOGY AND EVOLUTIONARY BIOLOGY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

OF THE SCHOOL OF SCIENCE AND ENGINEERING

OF TULANE UNIVERSITY

FOR THE DEGREE

OF

DOCTOR OF PHILOSOPHY

BY

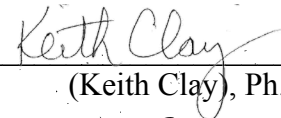


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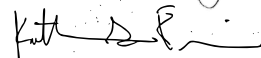
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## ABSTRACT

Tropical tree species' survival hinges on frugivore-mediated seed dispersal, affected by the fruiting neighborhood's dynamics. This study explores how spatial scales and frugivore groups impact dispersal in *Oenocarpus bataua*, a canopy palm. At local scales, heightened fruit density hinders flying dispersers due to competition, while aiding non-flying dispersers through facilitation. Landscape-scale fruit abundance encourages flying frugivores (facilitation), leaving non-flying dispersers unaffected. These findings emphasize frugivore functional groups and spatial scales as critical for understanding competition and facilitation in dispersal among conspecific fruiting trees.

Soil pathogens significantly impact plant survival, driven by resistance genes (R genes) inducing immune responses. In *Elaeis guineensis*, R gene diversity is well-studied, yet broader ecological and evolutionary aspects remain unclear. Here, targeted gene capture identifies 210 R gene regions in *O. bataua*, suggesting pathogen-mediated selection. Though adult R gene heterozygosity doesn't correlate with progeny survival, this approach underscores both benefits and limitations of exploring R genes in non-model species. This underscores the need to unravel genetic mechanisms governing plant-pathogen interactions in the wild.

Negative density dependence (NDD) and within-species specialization by soil pathogens shape forest community diversity. *Iriartea deltoidea* seedlings were examined under various soil conditions. Maternal source tree and soil source significantly impacted survival and performance. Distance between maternal tree and soil source affected survival, highlighting

NDD's role. Soil from conspecific adults and maternal trees yielded similar survival and performance. These findings underscore within-species specialization's influence on seedling recruitment, prompting further investigation into genotypic variation and soil microbial interactions in plant community dynamics.

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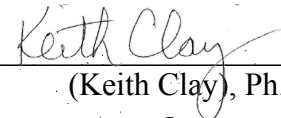


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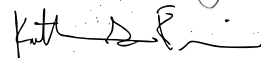
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I am lucky to have such amazing friends, finding each other in the various stages of our lives but still taking the time to support me despite their own busyness. True wealth is in friendship, and I am truly a rich man.

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## **Chapter 1: Effect of conspecific fruiting neighborhood on seed dispersal differs across frugivore functional groups and spatial scales**

### **INTRODUCTION**

More than 75% of tropical plant species rely on animals for dispersal of their seeds (Bascompte & Jordano, 2007; Howe & Smallwood, 1982). Animal-mediated seed dispersal is known to be advantageous, providing escape from high mortality near the maternal tree, colonization of uncompetitive environments, and directed dispersal into favorable microhabitats (Howe & Smallwood, 1982). As such, the quantity and quality of a plant's seeds that are dispersed by frugivores has important consequences for seedling survival and thus the fitness of that plant, as well as for emergent processes such as gene flow and community composition (Nathan, 2006; Schupp et al., 2010). For this reason, plants experience selection pressure for characteristics that maximize effective seed dispersal. Intrinsic characteristics of individual plants, including fruit color, nutritional value, accessibility, and crop size, have all been implicated in encouraging fruit removal by dispersal agents (Saracco et al., 2005; Schupp et al., 2019). Less well understood are the external characteristics that influence animal-mediated dispersal, including the fruiting neighborhood, defined here as the density of concurrently fruiting conspecific plant surrounding a focal fruiting plant at a given spatial scale.

The fruiting neighborhood could influence seed dispersal dynamics along the spectrum of plant-plant interactions, from neutral effects to the extremes of competition and facilitation (Schupp et al., 2019; Smith & McWilliams, 2014). Competition between adults within the fruiting neighborhood for animal dispersers may occur when fruit availability disproportionately exceeds the abundance of frugivores in the area, leading to reduced dispersal rates across

individuals (Manasse & Howe, 1983). Conversely, if increased fruit availability within the fruiting neighborhood disproportionately attracts frugivores, then facilitation between individuals may lead to increased dispersal rates (Sargent, 1990). Understanding how and when fruiting neighborhood has a competitive or facilitative effect on dispersal rates can help us to better predict seedling survival and maternal tree fitness. However, the magnitude and direction of these effects can be challenging to predict from the perspective of the individual, which benefits most from having the largest proportion of its fruit crop removed by high-quality frugivores (Schupp et al., 2017). Empirical evidence is mixed: depending on the system, high fruiting density has been shown to both attract (Blendinger et al., 2008; Blendinger & Villegas, 2011; Guerra et al., 2017; Morales et al., 2012; Sargent, 1990; Takahashi & Kamitani, 2004) or to reduce frugivore visits (Denslow, 1987; Jansen et al., 2014; Manasse & Howe, 1983; Moore & Willson, 1982; Moreira et al., 2017; Saracco et al., 2005; Smith & McWilliams, 2014).

These mixed results may be due, in part, to uncertainty about relevant spatial scales at which the fruiting neighborhood impacts seed dispersal dynamics. In contrast to the pollen neighborhood, which is well defined conceptually and mathematically (Crawford, 1984; Levin & Kerster, 1971), the fruiting neighborhood has no set definition. Various criteria have been used in selecting neighborhood scale across studies, including minimum distance among individual neighborhoods (Guerra et al., 2017); maximum observer visual range (Saracco et al., 2005); maximum frugivore visual range (Manasse & Howe, 1983); avian frugivore flight patterns (Carlo & Morales, 2008); or unstated factors (Moreira et al., 2017). As a consequence, the fruiting neighborhood has been described from as little as 5 m from a focal plant (Smith & McWilliams, 2014) to as much as 100 m (Moreira et al., 2017) in different studies. Few studies have

attempted to test neighborhood effects simultaneously at different spatial scales. Blendinger & Villegas (2010) tested two neighborhood scales (400 m<sup>2</sup> and 1,600 m<sup>2</sup>) chosen based on their plot size, and Carlo & Morales (2008) evaluated 100 radii between 1 and 100 m based on correlations between fruit density and bird flight distances in simulations and with field data. However, aside from these studies, the effect of the fruiting neighborhood at landscape scales on seed dispersal rates remains largely unexplored.

Another point of uncertainty concerns how different functional classes of frugivores might respond to the surrounding fruiting neighborhood. Many fruiting tree species support diverse frugivore communities (Stevenson et al., 2015), but efforts to concurrently measure the relevant scale of neighborhood effects on multiple frugivore functional groups are lacking, in part due to the difficulty in assigning dispersal events to distinct frugivore species. Avian dispersers are predominantly the sole functional group considered (Blendinger et al., 2008; Blendinger & Villegas, 2011; Carlo & Morales, 2008, 2008; Guerra et al., 2017; Morán-López et al., 2015; Pizo & Almeida-Neto, 2009; Saracco et al., 2005; Sargent, 1990; Smith & McWilliams, 2014; Takahashi & Kamitani, 2004). This represents an important knowledge gap as dissimilar functional groups of frugivores likely respond to neighborhood effects differently at varying spatial scales (García & Ortiz-Pulido, 2004). For example, large flying dispersers (e.g., toucans, hornbills), as well as large terrestrial or arboreal dispersers (e.g., tapirs, monkeys), tend to have large home ranges and may track fruiting resources across landscape-level spatial scales (Gleditsch et al., 2017; Graham, 2001; Hampe, 2008; Holbrook, 2011; Holbrook et al., 2002; Moreira et al., 2017; Wotton & Kelly, 2012). In contrast, smaller terrestrial seed dispersers (e.g., ants, smaller mammals, and smaller flightless birds) typically have more restricted home

ranges, and thus might be expected to respond to more localized fruiting neighborhoods, if at all. At present, however, the literature contains few direct tests of these expected differences in spatial scale sensitivities by different functional classes of disperser.

For these reasons, a concurrent assessment of how different spatial scales of the fruiting neighborhood shape seed dispersal rates by different functional classes of dispersal agents would advance our understanding of how fruiting neighborhood influence dispersal dynamics. In particular, it would shed light on the key question of how fruiting neighborhood densities at different spatial scales may contribute to facilitation of vs. competition among conspecific plants for different functional groups of seed dispersal agents. We address this knowledge gap by relating fruit removal to fruiting neighborhood at local and landscape scales for *Oenocarpus bataua*, a widespread Neotropical canopy palm species that produces large-seeded fruits consumed by multiple frugivore species (Goulding & Smith, 2007). To do so, we combined four years of fruit removal data from motion-activated cameras with detailed phenology data to assess the spatial scales at which the fruiting neighborhood impacts dispersal rates by each of two functional groups of dispersal agent, and what the direction and magnitude of these impacts are. We hypothesized that fruiting neighborhood will asymmetrically influence dispersal by different functional groups at varying spatial scales. We predicted that flying animal dispersal rates will be most sensitive to and increase with fruiting density at the landscape scale and that small non-flying animal dispersal rates will be most sensitive to and increase with fruiting density at the local scale.

## **MATERIALS & METHODS**

## Study system

Data collection was conducted at the Bilsa Biological station (hereafter BBS; 79° 45'W, 0°22'N; 330-730m elevation, 2-3 m precipitation annually). Located within the Mache-Chindul Reserve in Northwestern Ecuador, BBS is comprised of 3500 ha of primary and secondary humid rainforest in the Chocó biogeographic region. The Chocó biogeographic region is a global hotspot for conservation for its exceptional biodiversity and high rates of plant and animal endemism (Myers et al., 2000). This study was conducted within a 130 ha long-term study plot within BBS, where we have previously surveyed, identified, and geolocated all 181 adults of *O. bataua* (Browne et al., 2018; Mahoney et al., 2018). The study plot is bounded on the north, west, and south by continuous forest similar to that found in the study plot, but the eastern edge abuts farm and pasture lands. Hunting incursions into our study site may have reduced the populations of non-flying dispersers, particularly in terrestrial mammals, and large terrestrial frugivores (e.g., tapir) are not thought to be present.

*Oenocarpus bataua* Mart. var. *bataua* (Arecaceae) is a long-lived, hyper-abundant, monoecious, insect-pollinated canopy palm, that is widely distributed across lower to mid-range elevation forests in tropical South America (Henderson, 1995; Ter Steege et al., 2013). *Oenocarpus bataua* adults grow to 20-40 m in height (Henderson, 1995). In Ecuador, *O. bataua*'s range comprises the Tumbes-Chocó biogeographic zone west of the Andes mountain range and the Amazonian forests east of the Andes (Escobar et al., 2018). In BBS, *O. bataua* is highly-outcrossed with infrequent selfing (Browne et al., 2018; Ottewell et al., 2012). During supra-annual cycles of fruiting, *O. bataua* produces one or more infructescences of thousands of fleshy fruits, each containing one large seed covered by a thin, fatty aril (Ramirez-Parada et

al., 2020). *Oenocarpus bataua* supports a large frugivore community, but seed size limits effective primary dispersal to large-bodied frugivores. Previous work at this study plot using human observations identified long-wattled umbrellabirds (*Cephalopterus penduliger*), toucans (*Ramphastos* spp.) and red-tailed squirrels (*Notosciurus granatensis*) as dispersers of *O. bataua* fruits directly from the infructescence in the canopy (i.e., primary dispersal; Mahoney et al., 2018), but removal of fruits from the forest floor beneath infructescences (i.e., secondary dispersal) has not previously been documented in this study area. Large rodents, primates, and ungulates are major dispersers of *O. bataua* in other systems (Bodmer, 1991; Franco-Quimbay & Rojas-Robles, 2017; Peres, 1994, 2000b; Rojas-Robles et al., 2012; Stevenson, 2005).

### **Frugivore observations**

Between July of 2016 and May 2018, we used monthly phenological data collected on all 181 *O. bataua* adults in the 130-ha study plot to identify every adult with mature fruit, and we randomly selected a subset of those adults (n=47) to receive camera traps (Strikeforce Pro, Browning Trail Cameras, Birmingham, USA). For each fruiting adult we monitored, we placed a camera either at the base of the fruiting adult to capture secondary dispersal, or in the canopy at the level of the infructescence to capture primary seed dispersal, or both, depending on camera availability and functionality (see below). Fruits of *O. bataua* are densely clustered in single infructescences measuring more than 2 m (Henderson, 1995) meaning that a single camera frame can effectively capture all primary dispersal events. Ground cameras were placed to maximize the field of view of area directly beneath the infructescence to capture secondary dispersal events. In both cases, cameras were programmed to record 30 second videos with a five second delay. Each camera was active on average for 291.02 hours ( $\pm$  SD 71.37).

We inspected footage from each camera to ensure proper functionality. Footage from malfunctioning cameras, misplaced cameras, or from cameras otherwise not producing usable video were not considered for analysis. Each video was annotated with the frugivore species captured in the footage, number of animals seen, as well as any interaction with fruits. Mammal and bird species were classified to the species level using Ridgely & Greenfield (2001) for birds and Tirira (2017) and Urgilés-Verdugo & Viracocha (2018) for mammals. When possible, we classified rodents to species level, but small mice and rats unidentifiable by appearance or vocalization were broadly categorized as rodents. Owing to their similar coloration, appearance, ecological roles, and frequent lack of diagnostic vocalizations in our videos, we grouped Chocó toucans (*Ramphastos brevis*) and chestnut-mandibled toucans (*R. swainsonii*) as a single category at the genus level.

Animal interactions were subdivided into three categories: nibbling, in which frugivores chew on the flesh of the fruit without swallowing or carrying the fruit off-camera; ingestion, in which a fruit is swallowed; and removal, in which a fruit is removed away from the source tree outside of the wide viewing angle of cameras. We considered ingestion and removal of fruits as seed dispersal events and used these events to evaluate dispersal rates per hour for each camera at each tree for subsequent analyses, as described below. An animal species was classified as a seed disperser if we observed it ingesting or removing an *O. bataua* fruit at least once in our imagery. Because we placed camera traps in the canopy as well as on the ground, we were able to distinguish two distinct functional groups of dispersers, which we term 'flying' frugivores (all large birds, as no bats and no primates were observed removing fruits) and 'non-flying' frugivores (terrestrial birds and mid- to small-sized mammals; see Table 1). We

considered subdividing non-flying dispersers into terrestrial and arboreal but combined them because some species (e.g., squirrels) did not fit into this scheme, and because observations of arboreal, non-flying species were too scarce for modeling.

### **Fruiting neighborhood and habitat characterization**

Using phenology data for every adult *O. batava* tree in the 130-ha study plot, we counted the number of fruiting adults across the entire study plot surrounding each camera-equipped fruiting tree each month during the 22-month study period at multiple distance radii. To do so, we started at a 50 m radius area around the focal tree and increased by 50 m increments until 1500 m, the maximum distance between two adults within the study plot. Because the duration of our camera placements may not encompass the entire life of the infructescence, we also calculated the density of the fruiting neighborhood for a tree for a three-month period including the focal month plus one month before and after (Diaz-Martin & Karubian, 2021). As qualitatively similar results were obtained in both scenarios, we used only the focal month neighborhood in our models.

Habitat characteristics, including indices of prior disturbance, have previously been shown to explain variation in ecological processes within our study plot (Durães et al., 2013; Karubian et al., 2016; Lamperty et al., 2021; Mahoney et al., 2018). We selected four such previously significant variables to include in our models: canopy height, as measured with a range finder; percent of canopy cover taken with a spherical densiometer; number of trees of >50 cm diameter at breast height (DBH) within 20m of the focal tree, and the quantity of *Cecropia* spp. trees within 10 m of an adult. These habitat variables were dropped from our final models, due to lack of statistical significance and issues with model convergence.

## Statistical Analyses:

We used a paired two-sided T test on trees that simultaneously received a ground and an infructescence level camera to determine whether camera height influenced mean dispersal. Effect size was computed with Cohen's *d*. To determine the most informative scale for the local and landscape fruiting neighborhoods, we created a series of generalized linear mixed models fit with a Poisson distribution with dispersal rate as the response variable and each distance band of the fruiting neighborhood as predictors. Two more series of models were conducted with dispersal subdivided to flying dispersers and non-flying dispersers. We used an AIC-based model selection approach to determine at which spatial scale the density of the fruiting neighborhood best predicts dispersal. Each model included camera height as a covariate and the tree identification number as a random effect. To account for potential temporal autocorrelation, we attempted to include the date as a random effect, but ultimately removed it due to model convergence issues. As adult *O. batava* in our study site show spatial aggregation (Fig. 1), we tested the model residuals for spatial autocorrelation to determine if it was necessary to include correlation structures in our models to compensate (Bivand et al., 2013). Although we found no spatial autocorrelation in our residuals (Table S1), we limited our distance radii to 50m, 500m, 1000m, and 1500m to mitigate the potential for spatial autocorrelation.

We used generalized linear mixed models fit with a Poisson distribution using the package *glmmTMB* (Magnusson et al., 2020) to determine the relative effects of density of the fruiting neighborhood on frugivore dispersal rates. Using the results of the earlier model selection process, we used the distance radius that best explains non-flying dispersal for the

local fruiting neighborhood size and the distance radius that best explains flying dispersal as the landscape neighborhood size. Another predictor in the model included the height of camera placement (high vs. low). As a result of the sporadic and patchy timing of fruiting between trees, the local fruiting neighborhood density was added as a zero-inflation parameter because for many focal trees there were no fruiting conspecifics within the fruiting neighborhood ( $n = 93$ ) (Perumean-Chaney et al., 2013). Individual tree ID was included as a random effect to account for spatial autocorrelation. As dispersal rates could have non-linear relationships with neighborhood density, we also fitted the model with a quadratic function. The quadratic model was qualitatively similar, but linear models best fit the data and are reported here.

## RESULTS:

From July 2016 to May 2018, we placed cameras on 47 unique adults of *O. batuaa*. Cameras were deployed for a total of 43,944 camera trap hours, with a mean sampling period lasting for 291 hours ( $\pm 71.4$  SD). Ground-level camera hours (25,968 hours, mean  $\pm$  SE:  $298 \pm 74.2$ ) exceeded those at the infructescence-level (17,976 hours, mean  $\pm$  SE:  $281 \pm 66.6$ ).

Out of 5,553 videos in which animals appear, 1,937 (~35%) contained known seed dispersers of *O. batuaa* (i.e., species that we observed ingesting or removing seeds at least once, Fig. 2). In total, we recorded 1,464 dispersal events: 943 or ~64% at the infructescence level and 521 or ~36% at ground level. Cumulative dispersal rates did not differ between infructescence and ground-level cameras ( $t_{49}=1.19$ ,  $p=0.238$ ), but dispersal rates by non-flying dispersers were higher at ground-level cameras ( $t_{49}=-4.95$ ,  $p<0.001$ ,  $d=-0.7$ ) and dispersal rates by flying dispersers were higher at infructescence level cameras ( $t_{49}=4.17$ ,  $p<0.001$ ,  $d=0.589$ ).

Our suite of known seed dispersers comprised 15 total species, 5 flying and 10 non-flying dispersal agents (Table 1). Toucans (*Ramphastos spp.*) were the most frequent dispersers (724 dispersal events) at the infructescence-level, while myomorphid rodent spp. were the most frequent dispersers (197 dispersal events) at the ground-level.

#### *Selection of relevant spatial scale size*

Model selection to determine the most appropriate spatial scales found that both 1,000 m and 1,500 m fruiting neighborhood radii around focal trees explained overall dispersal rates nearly equally well (AIC=1,969.4, SE=1.13,  $p < 0.001$ , Table 2). Within functional groups, both the 1,000 m and 1,500 m models explained dispersal rates equally well for flying dispersers (AIC= 984.3, SE=2.51,  $p = 0.03$ ), whereas the 50m model best explained dispersal rates by non-flying frugivores (AIC= 983.4, SE= 1.32,  $p = 0.03$ , Table 3).

#### *Impact of fruiting neighborhood scale on dispersal rates of flying vs. non-flying dispersal agents*

Flying and non-flying frugivores responded to fruiting neighborhoods at different spatial scales, in different ways (Fig. 3). Increasing fruit availability at the local scale (50 m around a fruiting tree) was associated with marked decrease in dispersal rates by flying frugivores ( $z = -3.95$ , SE=.26,  $p < 0.001$ , Fig. 3a) and a weaker response of increased dispersal rates by non-flying frugivores ( $z = 2.03$ , SE=.30,  $p = 0.04$ , Fig. 3c). At the landscape scale (1,500 m around a focal tree), increased fruit availability was associated with increased dispersal rates by flying frugivores ( $z = 5.13$ , SE=.01,  $p < 0.001$ , Fig. 3b), but there was no effect on dispersal rates by non-flying frugivores ( $z = 0.15$ , SE=.01,  $p = 0.88$ , Fig. 3d).

## **DISCUSSION**

Although it is widely recognized that fruiting neighborhood effects are likely to be an important driver of fruit removal rates and seed dispersal, the magnitude and direction of these impacts varies dramatically between systems, and our understanding of what drives this variation is incomplete. Our study provides two important advances that help to fill this knowledge gap. First, we considered multiple radii spanning local (50 m radius) to landscape (1,500 m) scales whereas most previous studies have investigated these dynamics at a single spatial scale. Second, in contrast to earlier work that focuses largely on one or a few species of avian dispersal agents (but see Prasad & Sukumar, 2010), we concurrently tested the effect of fruiting neighborhood density on two distinctive functional groups: flying and non-flying dispersers. We found that neighborhood effects on dispersal rates varied in relation to both spatial scale and disperser functional group: a higher density of fruiting conspecifics at the local scale was associated with decreased dispersal rates among flying dispersers but higher dispersal rates among non-flying dispersers, whereas a higher density of fruiting conspecifics at the landscape scale was associated with increased dispersal rates by flying dispersal agents and no detectable effect on non-flying dispersal agents. Our results highlight the importance of context when quantifying neighborhood effects and suggest that considering the movement and foraging ecology of dispersers may be key to understanding variation in fruiting neighborhood dynamics.

At the local scale, flying dispersal rates decreased with increasing neighborhood density, consistent with the competition hypothesis for this functional group at this spatial scale. In areas of high conspecific density, competition among individual trees for flying dispersers may reduce dispersal rates for any specific individual across the neighborhood. Previous work has found that the effect of competition is pronounced in large-seeded plants that depend on few

large-bodied frugivores (Moreira et al., 2017) and in plants with low crop sizes (Blendinger et al., 2008; Prasad & Sukumar, 2010). The large-seeded *O. bataua*'s apparent reliance on large-bodied dispersers at our study site aligns with the first of these expectations, but *O. bataua*'s large crop sizes fail the other condition. Conversely, competition may also be exacerbated in plants with large crop sizes in high neighborhood densities, resulting in high fruit availability leading to frugivore satiation and a corresponding reduction in dispersal rates (Prasad & Sukumar, 2010). Flying frugivores in videos frequently "tested" many fruits from a single tree before ingestion, potentially spending less time foraging at other trees. Decreases in frugivory can contribute to dispersal limitation, or the failure of seeds of a certain species to reach an available site by either lower fecundity or inadequate distribution of propagules (Terborgh et al., 2011, 2019). For a fecund species like *O. bataua*, a decrease in dispersal by flying frugivores at local scales may limit distribution of seeds, leading to dispersal limitation for individual trees and ultimately fewer long-range dispersal events and lower recruitment rates.

In contrast, non-flying dispersal rates increased with neighborhood density at the local scale, supporting the facilitation hypothesis for this functional group at this spatial scale. This result is consistent with smaller-bodied mammals and terrestrial bird species tracking fruiting resources at short distances (Aliaga-Rossel et al., 2008; Stevens et al., 2014). Odors released by fermentation intensify under high fruiting densities and attract peccaries, coatis (*Nasua nasua*), and various rodent species, including pacas and agoutis (Sanchez-Cordero & Martinez-Gallardo, 1998). Smaller mammals will also selectively forage in denser fruiting patches to minimize predation risk and costs associated with searching and processing of fruits (giving-up density, Brown 1988). Many of the rodent species we observed (e.g. *Notosciurus granatensis*,

*Dasyprocta punctata*) have been found to be effective dispersers of *O. bataua* in more disturbed systems (Rojas-Robles et al., 2012). Rodent species have been observed moving *O. bataua* seeds in ~20% of all interactions, with occasional dispersal distances greater than 50m (Rojas-Robles et al., 2012). Rodents also scatter-hoard seeds into favorable microhabitats for seedling establishment and hastened germination by removing the mesocarp of the seed (Rojas-Robles et al., 2012), a behavior also seen in *Dasyprocta punctata* in other systems (Hirsch et al., 2012). Thus, an increase in non-flying dispersers because of increased neighborhood density at the local scale could replace at least part of the dispersal services lost for individual trees in the corresponding decrease in flying dispersers, though presumably with shorter dispersal distances. This observation supports previous work on genetic structure of *O. bataua* seedlings in our system, finding that genetic kinship of seedlings was high between 10m distance intervals but rapidly decreased at 50m, 100m, 150m intervals (Browne et al., 2018).

At the landscape scale, flying dispersal rates were positively related with fruiting neighborhood density, consistent with the facilitation hypothesis for this functional group at this spatial scale. This finding aligns with other studies showing that multiple co-fruiting trees in a large area may attract flying dispersers that appear to track resources at a regional scale. For example, toucans have by far the highest proportion of dispersal of all dispersers in this study (Table 1) and will travel long distances in extensive home ranges in search of fruits (Holbrook, 2011). The facilitative effect of neighborhood density at the landscape scale may be explained by *O. bataua*'s phenology. Reproductive structures of *O. bataua* require ~2 years of development (Rojas-Robles & Stiles, 2009), leading to asynchronous fruiting in populations of *O. bataua* with peaks occurring every ~1.5 years (Ramirez-Parada et al., 2020). During our study

period, flying dispersal rates peaked within a few months of peaks in neighborhood fruiting density (figure 1). This is consistent with the theoretical model proposed by Gleditsch et al. (2017), which suggested that facilitative neighborhood effects are temporally linked to resource availability and frugivore abundance. While facilitation of frugivore dispersal has been observed in other systems, our results differ in several important ways. Facilitative neighborhood effects were generally found either in plants with relatively small crop sizes or at small neighborhood scales (Sargent 1990, Takahashi & Kamitani 2004, Blendinger *et al.* 2008, Pizo & Almeida-Neto 2009, Blendinger & Villegas 2011, Morán-López *et al.* 2015, but see Guerra *et al.* 2017). Flying dispersers generally, and toucans and long-wattled umbrellabirds specifically, accounted for ~97% of all observed seed ingestions. Seeds ingested by these large birds can be transported significant distances before being regurgitated, and represent a higher probability of long distance seed dispersal into favorable conditions (Karubian et al., 2012; Kays et al., 2011). Moreover, previous work in our study area has shown that these dispersers likely promote genetically-diverse seed pools of *O. batava* across the landscape (Browne et al., 2018).

These insights into fruiting neighborhood effects across spatial scales and functional groups were made possible by phenological information on each adult individual of our focal species in our study area, which is relatively rare among published studies. Carlo & Morales (2008) found that the fruiting neighborhood effects in field studies decreased removal rates (i.e., competition) at radii from 1 m to 100 m, with the strongest effect at 45 m. The start of these radii were located in the center of 10 separate subplots in which all fruiting adults of *Cestrum diurnum* were counted. Blendinger and Villegas (2011) compared two neighborhood size radii (20 m and 40 m) centered in subplots containing a fruiting adult of *Eugenia uniflora*,

finding a marginal positive relationship (i.e., facilitation) at the 20 m radii. Our study differs and builds on these in several important ways. First, we established the relevant neighborhood scale using individual trees as the central point of each radii, rather than the center of subplots. As individual trees are the focal unit on which neighborhood effects influence fruit removal and seed dispersal (Schupp et al., 2019), using individuals as the focus logically should be a more accurate means of determining relevant neighborhood scales. Second, we tested a much wider range of radii (up to 1500 m). Future studies will benefit from incorporating fruit availability measures at broad spatial scales; drones and remote sensing increasingly provide cost-effective ways to gather these data.

Comparison of our results with those of an earlier study by Mahoney et al. (2018) with direct observation during daylight hours by researchers (i.e., instead of camera traps) in the same study site highlights the importance of sampling effort and methodology in capturing neighborhood effects: the use of camera traps allows for more detections, particularly among nocturnal dispersers. Direct, daytime observations on primary dispersal from infructescences of *O. bataua* by Mahoney et al. (2018) recorded 256 dispersal events (~0.66 events per observer hour) by five bird species and one mammal species over the span of seven years (2009-2016). Utilizing camera traps over a two-year period (2016-2018), we recorded 976 primary dispersal events (~0.04 events per observer hour) directly from the infructescence by five bird species and three species of mammal. We observed all the same disperser species as Mahoney et al. (2018), with the exception of a single parrot species (*Pionus chalcolpterus*) as well as six additional bird species. Relative to direct observation, the camera traps recorded slightly lower dispersal proportions for toucans foraging at the infructescence level (57.7% vs.

59.8%) and substantially lower proportions for long-wattled umbrellabirds (8.9% vs. 40.2%). This disparity may be because umbrellabird males forage near leks (display sites for females), and many of the trees observed by Mahoney et al. (2018) were proximate to lek sites. In contrast, we recorded a much higher rate of removal by squirrels foraging at the infructescence level (*N. granatensis*; 8.4% vs .4%), perhaps due to the fact that squirrels may be more sensitive to human observer presence than some other species.

Because we placed camera traps on the forest floor, we also recorded additional mammal species engaged in secondary dispersal of fruits on the forest floor. Three bird species (*Ramphastos* spp., *C. penduliger*, *Penelope ortonii*) and one mammal species (*N. granatensis*) were seen engaging in both primary and secondary dispersal. Additionally, our cameras captured important nocturnal dispersers that went undetected by Mahoney et al. (2018): in fact, 55% of the disperser species identified were nocturnal although these species cumulatively accounted for only ~20% of all dispersal events we recorded. Interestingly, we did not capture any primates, bats or ungulates as dispersers, despite their prominence as seed dispersers of *O. bataua* in Amazonia (Bodmer, 1991; Karubian et al., 2015; Peres, 2000a; Stevenson et al., 2015). This may reflect local extirpations or population declines of ungulates via hunting in our project area, and the fact that spider monkeys (*Ateles* spp.), a major consumer of *O. bataua* fruits in the Amazon, are absent from the site. Disperser communities for *O. bataua* may vary depending on the study site: for example, Rojas-Robles et al. (2012) observed seed dispersal using camera traps in the Colombian Andes, finding that squirrels were the most prolific dispersers with a single bird species and no primates. Thus, our observed

disperser communities will likely differ from communities found in other systems with *O. bataua*.

We demonstrate that the fruiting neighborhood of *O. bataua*, through facilitation and competition, explains patterns of frugivore visitation across separate functional groups at varying spatial scales. It is striking that we were able to detect these relationships without taking fruit availability of other tree species into account, as all the frugivore species we recorded are generalists that feed from many trees besides *O. bataua*. This may be explained in part by the fact that *O. bataua* is considered a keystone species that has at least some individuals bearing thousands of energy-rich fruits year-round (Ramirez-Parada et al., 2020), suggesting it might have a disproportionate trophic influence relative to its abundance (Diaz-Martin et al., 2014). Future work in this system might explore impacts of fruit availability at the community level on seed dispersal of *O. bataua* to gain a more nuanced understanding of these dynamics. Direct tracking of frugivores or use of molecular markers to determine impacts on seed movement and deposition, as well as tracking seeds to determine seed fate, represent other exciting avenues for future research. More broadly, in whatever system researchers are working, spatial scale and functional group(s) should be carefully considered when studying neighborhood effects.

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**Author Contributions:**

KN, LB, ZDM, and JK designed research. KN, JO, DC, and LB performed research. KN, LB, and ZDM analyzed data. KN, LB, ZDM, and JK wrote the paper.

**Data Accessibility:**

We intend to archive our data on the Dryad Digital Repository ([datadryad.org](http://datadryad.org)).

Tables and Figures

**Table 1:** Vertebrate frugivores recorded dispersing fruits of the canopy palm *Oenocarpus bataua* in northwest Ecuador using camera traps at canopy and ground levels, divided into flying dispersers and non-flying dispersers.

Species	Scientific Name	Class	Functional Group	Proportion of Total Dispersal Events (%)	Dispersals per hour x 100
Toucan spp.	<i>Ramphastos</i>	Aves	Flying	49.59	1.652
Long-wattled umbrellabird	<i>Cephalopterus penduliger</i>	Aves	Flying	8.88	0.296
Oilbird	<i>Steatornis caripensis</i>	Aves	Flying	2.46	0.082
Southern mealy parrot	<i>Amazona farinosa</i>	Aves	Flying	0.48	0.016
Baudó guan	<i>Penelope ortonii</i>	Aves	Flying	0.41	0.014
Red-tailed Squirrel	<i>Notosciurus granatensis</i>	Mammalia	Non-flying	17.01	0.567
Rodent spp.	--	Mammalia	Non-flying	13.46	0.448
Central American agouti	<i>Dasyprocta punctata</i>	Mammalia	Non-flying	3.01	0.100
Lowland paca	<i>Cuniculus paca</i>	Mammalia	Non-flying	2.12	0.071
Tome's spiny-rat	<i>Proechimys semispinosus</i>	Mammalia	Non-flying	1.64	0.055
Brown four-eyed opossum	<i>Metachirus nudicaudatus</i>	Mammalia	Non-flying	0.27	0.009
Collared peccary	<i>Pecari tajacu</i>	Mammalia	Non-flying	0.27	0.009
Kinkajou	<i>Potos flavus</i>	Mammalia	Non-flying	0.20	0.007

Species	Scientific Name	Class	Functional Group	Proportion of Total Dispersal Events (%)	Dispersals per hour x 100
South American coati	<i>Nasua nasua</i>	Mammalia	Non-flying	0.14	0.005
Common opossum	<i>Didelphis marsupialis</i>	Mammalia	Non-flying	0.07	0.002

**Table 1:** Proportion of dispersal is the observed number of dispersal events by a given species divided by the total dispersal events (N=905) by all species. Dispersals per day is the observed number of dispersal events by a given species divided by the total camera days (N=1831) in the study period.

**Table 2:** Summary of results of  $\Delta AICc$  approach using model comparison to determine appropriate fruiting neighborhood (FN) radii for the local and landscape spatial scales.

	FN	dAICc	estimate	std. error	statistic	p value
Combined Disperser Fruit Removal	1000	0.00	-0.38	0.18	-2.14	<b>0.03</b>
	1500	0.00	-0.38	0.18	-2.14	<b>0.03</b>
	500	8.43	-0.24	0.19	-1.30	0.19
	50	17.91	0.14	0.17	0.83	0.41
	NULL	993.46	2.85	0.02	125.45	0.00
Flying Disperser Fruit Removal	1000	0.00	-0.89	0.28	-3.19	<b>0.00</b>
	1500	0.00	-0.89	0.28	-3.19	<b>0.00</b>
	500	7.69	-0.69	0.31	-2.27	0.02
	50	17.93	-0.13	0.24	-0.57	0.57
	NULL	900.58	2.88	0.03	87.17	0.00
Non-flying Disperser Fruit Removal	50	0.00	-0.54	0.21	-2.55	<b>0.01</b>
	1000	1.64	-0.48	0.28	-1.71	0.09
	1500	1.64	-0.48	0.28	-1.71	0.09
	500	1.96	-0.57	0.26	-2.18	<b>0.03</b>
	NULL	422.33	2.52	0.03	80.15	0.00

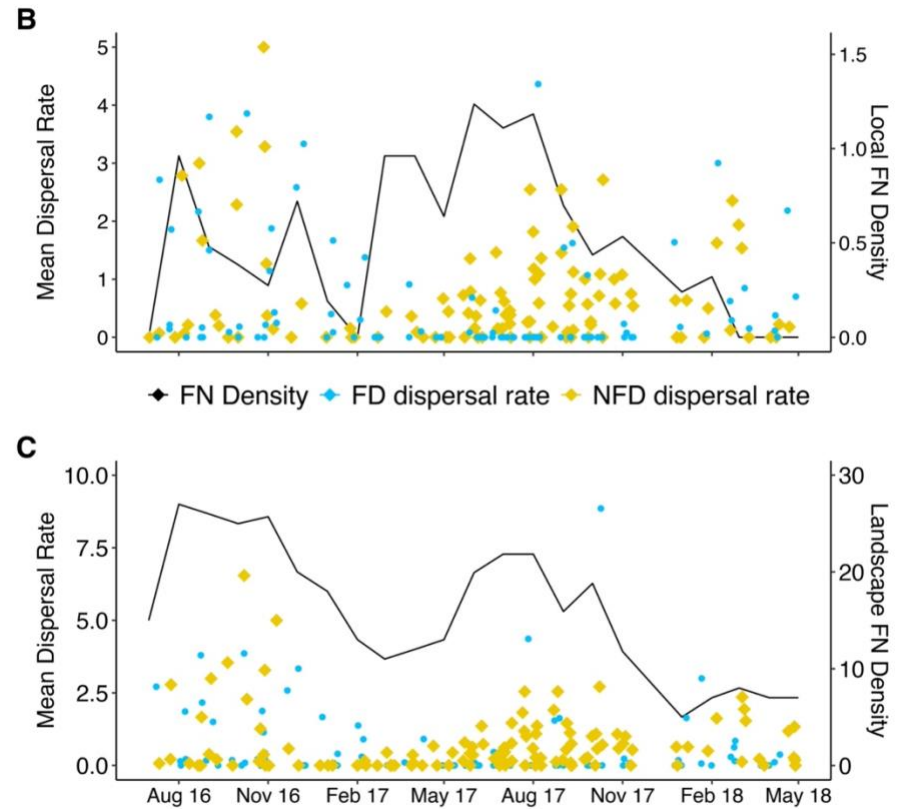
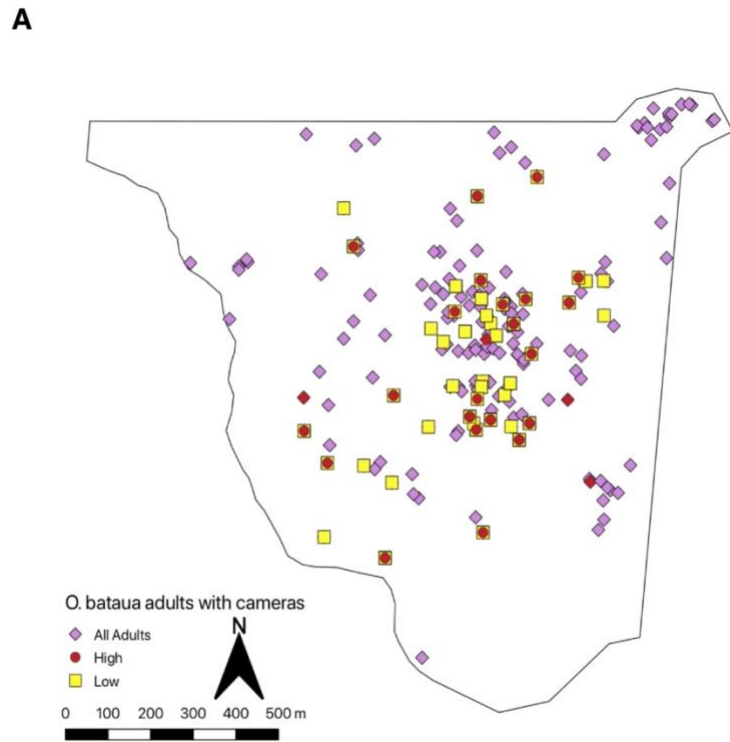
**Table 2:** The 1,000 m and 1,500 m models were identical and the highest quality models (i.e., lowest dAICc) for both cumulative and flying dispersal rates. The 50 m model was the highest quality model in predicting non-flying dispersal rates.

**Table 3:** Model coefficients for the best-fitted generalized linear mixed models for *Oenocarpus bataua* seed dispersal rates cumulatively, for flying dispersers only, and non-flying dispersers only.

Predictors	Cumulative Dispersal				Flying Dispersal				Non-Flying Dispersal			
	Estimate	SE	z value	p	Estimate	SE	z value	p	Estimate	SE	z value	p
Intercept	-0.49	0.19	-2.64	<b>0.01</b>	-0.81	0.29	-2.81	<b>&lt;0.001</b>	-0.75	0.30	-2.50	<b>0.01</b>
FN 50 m	-0.17	0.16	-1.06	0.29	-1.03	0.26	-3.95	<b>&lt;0.001</b>	0.62	0.30	2.03	<b>0.04</b>
FN 1500 m	0.04	0.01	5.14	<b>&lt;0.001</b>	0.05	0.01	3.89	<b>&lt;0.001</b>	0.01	0.01	1.00	0.32
Height [low]	-0.54	0.05	-10.31	<b>&lt;0.001</b>	-1.98	0.22	-8.86	<b>&lt;0.001</b>	0.20	0.12	1.66	0.10
<b>Zero-Inflated Model</b>												
Intercept	-1.02	0.22	-4.56	<b>&lt;0.001</b>	0.12	0.24	0.53	0.60	-0.06	0.20	-0.31	0.76
FN 50 m	-0.18	0.21	-0.85	0.39	0.27	0.22	1.21	0.22	-0.22	0.17	-1.28	0.20
<b>Random Effects</b>												
$\sigma^2$	0.71				1.70				0.55			
$\tau_{00}$	0.78 <sub>Tree</sub>				1.55 <sub>Tree</sub>				0.72 <sub>Tree</sub>			
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.094 / 0.567				0.287 / 0.628				0.025 / 0.576			

1 **Table 3:** All models used as predictor variables the number of fruiting adults within a 50m radius (FN 50 m), the number of fruiting  
2 adults within a 1500m radius (FN 1500 m), and the camera height (low: ground-level vs. high: infructescence-level). As models were  
3 overdispersed from zero-inflation, FN 50 m was included as a zero-inflation term. The tree identifier was used as a random effect  
4 and was sufficient to compensate for spatial autocorrelation. Fixed predictors are log rates for the intercepts or log rate ratios for all  
5 other variables. Bold  $p$  values indicate values  $<0.05$ . Flying dispersal rates were negatively associated with an increase in FN 50 m  
6 and positively associated with an increase in FN 1500 m. Non-flying dispersal rates were and positively associated with an increase in  
7 FN 50 m.

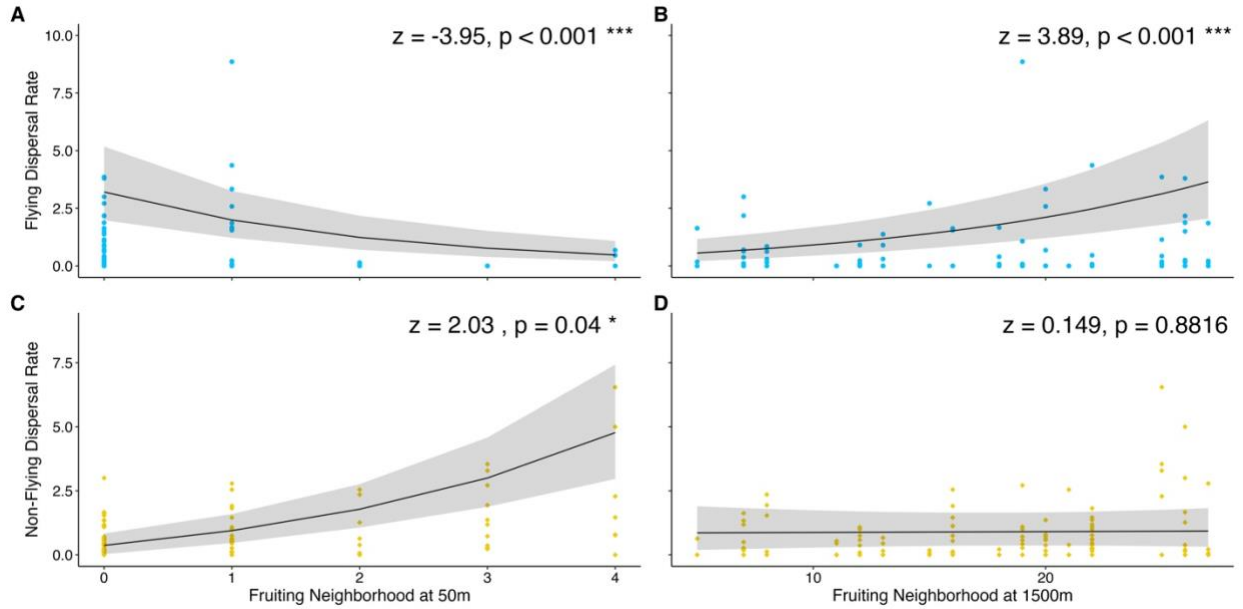
8 **Figure 1: (A)** Map of the study plot in northwest Ecuador with locations of adult *O. bataua* trees, and the locations of placed  
 9 cameras. High cameras were placed at canopy height to observe Infructescences, while low cameras were placed at ground level to  
 10 capture secondary dispersal. **(B)** Relationships between local fruiting neighborhood density, flying dispersal rates, and non-flying  
 11 dispersal rate at a given month during the study period. **(C)** Relationships between landscape fruiting neighborhood density, flying  
 12 dispersal rates, and non-flying dispersal rate at a given month during the study period.



**Figure 2:** Examples of frugivores interacting with fruit of *Oenocarpus bataua* captured at infructescences and at ground level. Species shown are **(A)** toucan spp. (*Ramphastos* spp.); **(B)** long-wattled umbrellabird (*Cephalopterus penduliger*); **(C)** Central American agouti (*Dasyprocta punctata*); and **(D)** red-tailed squirrel (*Notosciurus granatensis*).



**Figure 3:** Plots demonstrating marginal effects of covariates on predicted dispersal rates. Plots A and B show the marginal effects of local fruiting neighborhood density and landscape fruiting neighborhood density respectively for flying frugivores. Plots C and D show the marginal effects of local fruiting neighborhood density and landscape fruiting neighborhood density respectively for non-flying frugivores.



## Chapter 2: A preliminary evaluation of the evolutionary and ecological dynamics of R genes in the tropical palm *Oenocarpus bataua*

### Introduction

The interactions between pathogens and their hosts play a fundamental role in driving ecological and evolutionary dynamics in most biotic systems. Along with other factors (e.g., environmental filtering, competition), soil pathogens are known to be significant agents of plant mortality, particularly at the seedling establishment stage and in the tropics (e.g., Bagchi et al., 2010, 2014; Bever et al., 2015; Burdon et al., 2006). There is increasing evidence that a group of genes termed resistance or ‘R genes’ may play a key role in mediating plant susceptibility to soil pathogens (reviewed in Ngou et al., 2022). Broadly speaking, different R genes enable plants to identify specific classes of pathogens, triggering an immune response once identification has occurred. R genes are well-studied in agricultural and model species, but several key aspects of their basic biology including intraspecific diversity, signatures of selection, and potential impacts on seedling survival remain understudied and poorly understood in wild systems.

Decades of work in model and agricultural systems have detailed that R genes in plants are primarily responsible for detecting and defending against pathogens such as fungi, oomycetes, viruses, bacteria, and some nematodes and insects (Gururani et al., 2012; Meyers et al., 2005; Stump et al., 2020). Plant immunity occurs in a two-tiered system, where disease resistance is initially provided by pattern recognition receptors (PRRs) located in the plant cell wall that detect infection and initiate an immune response. Adapted pathogens evade this basal immunity with effector proteins encoded by *AVR* (avirulence) genes. Thus, the second tier of immunity is provided by R genes, nucleotide-binding leucine-rich repeat receptors (NLRs),

which detect pathogen effectors and instigate an immune response (Gururani et al., 2012). In the simplest case, there is a gene-for-gene relationship between a plant R gene and a pathogen avirulence gene. The pathogen avirulence gene codes for a particular product that is recognized either directly or indirectly by receptors encoded by the corresponding plant R gene (Hammond-Kosack & Jones, 1997; Meyers et al., 1999). R genes are present in all land plants and hundreds of R genes have been identified in agricultural species (Baggs et al., 2017; Ngou et al., 2022). R gene diversity is not uniform: woody, longer-lived species (Baggs et al., 2017) and grasses like rice and wheat exhibit higher NLR diversity (Barragan & Weigel, 2021; Gong et al., 2022a). These differences may be attributed to the strength of pathogen pressure, with decreased burdens resulting in loss of relevant R genes (Barragan & Weigel, 2021; Gong et al., 2022b; Y. Liu et al., 2021).

In natural, as in agricultural systems, variation in R gene diversity is likely to influence the ability of individuals or populations to identify, respond to, and survive attack from pathogens. Existing work in wild systems has largely focused on congeners (Cao et al., 2020; Keepers et al., 2023; Stam et al., 2016; Winters, 2022) or progenitor species (Arora et al., 2019) of model or agricultural taxa with available reference genomes. Fewer studies have examined R gene diversity and evolution in *de novo* species. Previous work on wild systems has utilized either a transcriptomics approach (Marden et al., 2017), or via genome assembly followed by quantification of potential R genes through identification of tandemly duplicated genes (Sork et al., 2022). Because R genes are known to be highly conserved across plant species, genera, and even families (Meyers et al., 1999; Puch-Hau et al., 2016; Winters, 2022), targeted gene capture of R genes identified from more distantly related plant species whose genomes are mapped

may represent an alternative way to study R genes directly in non-model organisms. To the best of our knowledge, the efficacy of this approach has yet to be evaluated. As such, one goal of the current study is to evaluate an approach that leverages existing knowledge about R genes in agricultural and model organisms and applies it to non-model plants via targeted gene capture.

A second goal of the current study is to provide a preliminary characterization of R genes in a non-model species, with a focus on intraspecific diversity of R genes exhibited by individuals of a single population. Because there is strong selection pressure for pathogens to evade detection by plants, and for plants to maintain the ability to detect the ever-changing pathogens, we might expect balancing selection on R genes to maintain diversity on long timescales within a species. Similarly, we might also expect balancing selection for increased genetic diversity among conspecific individuals in a single population at ecological time scales. This is because a wide range of R gene alleles is likely to be necessary to respond to an ever-evolving array of pathogen enemies that generally have much shorter generation times than the plants they are attacking. Thus, individual plants with higher levels of R gene heterozygosity within a population might be expected to maintain superior immunity (i.e., by being able to detect and respond to a broader array of pathogens).

Our third goal was to provide a preliminary test of the degree to which R gene heterozygosity among individual adults might have a detectable impact on mean progeny survival. Using a transcriptomics approach that detected R proteins expressed in roots in response to differing soil and hormone treatments, Marden et al. (2017) provided initial empirical support for the idea that variation in R gene diversity may influence seedling survival among species. However, within-species assessments of the relationship between intraspecific

variation in R gene diversity and progeny survival are lacking. Using neutral microsatellite markers, our team has found evidence for an intraspecific 'rare genotype survival advantage' at the individual level in the palm *Oenocarpus bataua*, in that individuals with rare genotypes had a survival advantage over common genotypes, in both naturally dispersed (Browne & Karubian, 2018) and experimentally planted (Browne & Karubian, 2016) seedlings. But the degree to which among-individual variation in R gene heterozygosity per se might predict seedling survival in this and other natural systems remains unknown.

To fill these knowledge gaps, we provide a preliminary assessment of R gene evolutionary and ecological dynamics in *O. bataua*, a broadly distributed Neotropical canopy palm that is of considerable ecological and economic importance. First, we sought to establish the degree to which targeted gene capture of R genes from a palm model species (*E. guineensis*) could be used to study R gene diversity in *O. bataua*. Second, we sought to determine whether we could detect signatures of selection on R genes, as would be expected given the key role they are thought to play in immunity. Third, we sought to characterize patterns of R gene heterozygosity among adult *O. bataua* and assess whether among-individual variation in R gene heterozygosity may be associated with differences in progeny survival at ecological time scales.

## **Methods**

### *Study system*

Samples were collected at the Bilsa Biological station (hereafter BBS; 79° 45'W, 0°22'N: 330-730m elevation, 2-3 m precipitation annually). Bilsa Biological station, which is operated by the Jatun Sacha Foundation and located within the Mache-Chindul Reserve in the Chocó

biogeographic region, contains 3,500 ha of primary and secondary humid rainforest.

*Oenocarpus bataua* Mart. var. *bataua* (Arecaceae) is a long-lived, canopy palm, with adults reaching 40m in height (Henderson, 1995; Ter Steege et al., 2013) and is widely distributed on both sides of the Andes. Previous work in the area found that adult *O. bataua* display low intraspecies relatedness and low spatial genetic structure within our study site (Browne et al., 2018; Ottewell et al., 2012). Adults produce infructescences of thousands of large, single-seeded fruits, supporting a diverse assemblage of large-bodied frugivores involved in primary dispersal (Bodmer, 1991; Franco-Quimbay & Rojas-Robles, 2017; Peres, 1994, 2000; Rojas-Robles et al., 2012; Stevenson, 2005).

Karubian et al. (2016) planted 560 germinated seedlings whose seeds were initially collected from 30 adult *O. bataua* at a series of sites within BBS and monitored survival for five years via annual censuses to assess how environmental conditions influenced seedling survival, finding that canopy openness was the strongest predictor of survival. In a later study, Browne & Karubian (2016) leveraged this experimental design to assess the degree to which genetic rarity of seedling genotypes (assessed using neutral microsatellite markers) influenced probability of survival and found a strong relationship between rarity and survival similar in magnitude to canopy openness. Additionally, Browne & Karubian (2018) found that relative rarity of the maternal genotype predicted survival of seedlings as well as the seedling genotype.

#### *Sample collection and DNA extraction*

For the current study, we used leaf tissue collected directly from live leaves of 19 of the 30 *O. bataua* maternal trees used in Browne & Karubian (2016). These 19 *O. bataua* were selected *a priori* to include both the highest and lowest rates of seedling survival among 30

maternal trees used in the study. Upon collection, leaf tissues were placed in labeled envelopes and frozen prior to DNA extraction. We limited extraction to 16 maternal trees that represented the extremes of progeny survival outcomes. We extracted and purified DNA from the leaf tissue using the Qiagen DNeasy plant minikit (Qiagen, USA). Sample quality and concentration was tested using a spectrophotometer (Nanodrop 2000, Thermo Fisher Scientific, USA) with UV 260:280 ratios between 1.7 and 1.9 and with a Qubit 4 fluorometer (Invitrogen by Thermo Fisher Scientific, USA) machine respectively. We then shipped the DNA on dry ice to Arbor Biosciences for sequencing, library preparation, and RenSeq data acquisition (details below).

#### *Probe design and reference genome*

As *O. bataua* does not have a reference genome, we used the genome of *Elaeis guineensis* as our reference genome for target capture probe development. *Elaeis guineensis* is the most closely related organism to *O. bataua* with an annotated genome at the time of this study, also being a palm of the subfamily Arecoideae. Using the EG5.1 genome build of *E. guineensis*, we selected 210 R genes annotated as “Resistance genes” using the PalmExplore database (Chan et al., 2017). To ensure adequate sequencing coverage, we extracted just the coding sequences from the 210 annotated R genes for probe design. Coding regions < 10 bp in length were removed, and we added flanking sequences for coding regions  $\geq 10$  bp but  $\leq 79$  bp to bring probes to a total length of 80 bp, which is the target length for probe design under this protocol. This resulted in a set of 770 regions covering 434,548 bases in coding regions across the 210 R genes. To serve as a point of comparison with R genes, we chose a set of target regions for intergenic regions comparable in size, which we defined as any area of the genome

at least 10kb either upstream or downstream from an annotated gene of any type, which we assume for the purposes of the study are under neutral evolution. We also avoided N gaps in the genome assembly. Using bedtools (Quinlan & Hall, 2010), we randomly selected 770 intergenic regions, exactly similar in size to the selected R genes but non-coding, across the genome for subsequent comparisons.

The resulting 1,540 target loci, including both R genes and intergenic areas, were sent to Arbor Biosciences for bait design via their myBaits target enrichment via hybridization-based capture system (myBaits Custom DNA-Seq, Arbor Biosciences, USA). These target loci were subjected to Arbor's bait processing pipeline, which includes: replacing occurrences of N residues in stretches <10 with T residues, padding the front and back of a subset of sequences with T residues to meet length requirements for targets (80bp), and masking for repeat elements. These bait candidates were then BLASTed against the *E. guineensis* genome and further filtered based on their 'Moderate' filtration scheme, where some non-specific baits pass filtration. This resulted in a set of 13,482 custom baits. Sequencing was conducted on an Illumina NovaSeq 6000, with 150 bp paired-end read sequencing. Each sample was sequenced by Arbor BioSciences on two lanes to avoid lane effects.

### *Bioinformatics and data analysis*

Adaptors were trimmed from reads using Cutadapt (Martin, 2011). We used FastQC (Babraham informatics) to check read quality for all individuals and count total reads per individual to remove those with uncalled bases and with Phred scores lower than 20. The trimmed and filtered reads were aligned to the *E. guineensis* genome using BWA (H. Li & Durbin, 2009). We

identified duplicate reads using the MarkDuplicates tool in Picard (Broad Institute, 2019) and calculated coverage of each probe using bedtools. The GATK HaplotypeCaller and GenotypeGVCFs toolkits (Poplin et al., 2017) were used to identify SNPs in each of the 16 individuals. SNPs were then filtered with the GATK process 'VariantFiltration' for a minimum depth of coverage of 10, ReadPosRankSum  $\geq$  -8.0, QD  $\geq$  2.0, MQ  $\geq$  40.0, MQRankSum  $\geq$  -12.5, and GQ  $\geq$  20.0 using recommended values (De Summa et al., 2017). We further filtered SNPs with VCFtools (Danecek et al., 2011) to SNPs that had <50% missing data, and were bi-allelic.

Using the package PopGenome (Pfeifer et al., 2014) in R (R Core Team, 2022), we analyzed solely the R gene loci that mapped to known chromosomes and scaffolds. A similar process was unavailable for the intergenic loci as there were insufficient loci mapped to chromosome scaffolds. We then calculated Tajima's D, or a statistic which compares estimated nucleotide diversity of observed polymorphic sites against the estimated nucleotide diversity of the allele frequencies of polymorphic sites (Tajima, 1989). This statistic is commonly used to determine if a population faces selective pressure.

We calculated separate measures of individual-level heterozygosity (*i.e.*, the number of loci heterozygous for an individual / the total number of loci sequenced) for SNPs located within R gene targets and for intergenic targets using the 'snpR' package (Hemstrom & Jones, 2023). We used a paired non-parametric Wilcoxon Rank Sum Test to test for statistically significant differences in individual heterozygosity between R gene targets and intergenic targets, using the individual maternal tree as the pairing factor. We then tested the relationship between average individual heterozygosity against the proportion of seedling survival of

progeny for each individual from Browne and Karubian's (2016) field-based experiment (see above) separately for R gene targets and intergenic targets using Spearman's correlation in R.

## Results

### *Sequencing and mapping summary*

Across the 16 individuals sampled, we sequenced a total of 168,823,448 bases (range: 5,969,330 – 15,305,384 per sample). Mapping rates to the *E. guineensis* genome were 58.1% on average (range: 55.1% - 61.3%). Average depth of coverage for R gene sequences (112x) was substantially higher than for intergenic regions (27.2x), however the maximum depth of coverage was higher for intergenic regions (42,922x) than for R genes (3,383x). Approximately one-third (37%) of target intergenic regions had 0x coverage, while this number was much lower for R gene targets (8.4%). Filtering SNPs based on sequencing quality and missing data thresholds resulted in 1,625 high quality SNPs within 37/770 target intergenic regions and 21,305 SNPs within 525/770 target R gene regions.

We assessed Tajima's D for each R gene locus against a neutral simulation, finding a broad range of values centered around a mean of  $1.15 \pm 1.32$  for R genes and a narrower range for simulated values (mean:  $-0.09 \pm 0.45$ ) (Fig. 1). When Tajima's D is greater than 0, as we observed for R genes, few or no rare alleles are present indicating balancing selection or recent population decrease (values of 0 indicate that the population is evolving randomly with no evidence of selection, and negative values indicate an abundance of rare alleles, implying a recent population expansion and positive selective pressure, i.e. selective sweep) (Carlson et al., 2005).

Individual-level heterozygosity was significantly higher for R genes compared to intergenic regions (Paired Wilcoxon Rank Sum Test,  $V = 0$ ,  $P < 0.001$ , SI Table 1, Fig. 2a). Maximum and minimum individual heterozygosity at R genes were 0.35 to 0.327 compared with 0.126 to 0.090 for intergenic regions. Leveraging a field-based experiment to evaluate the degree to which diversity of maternal trees predicted seedling survival, we found no relationship between progeny survival with maternal tree heterozygosity at intergenic targets (Spearman's  $\rho = -0.035$ ,  $P = 0.896$ ) or R gene targets (Spearman's  $\rho = 0.07$ ,  $P = 0.794$ ) (Fig. 2b).

## Discussion

In the current study, we provide a preliminary evaluation of R gene evolutionary and ecological dynamics in the Neotropical canopy palm *O. bataua* by using targeted gene capture of R genes previously identified in the model species, *E. guineensis*. Our basic goal was to assess whether this approach would produce meaningful results and, if so, to examine selection strength on R genes and whether we could detect an impact of inter-individual differences in R gene heterozygosity on progeny survival. Target capture probes developed from *E. guineensis* successfully identified a 14-fold greater number of R gene regions vs. intergenic regions in our study species, validating the efficacy of this approach and highlighting the degree to which R genes are conserved across plant species. Similarly, we detected 13 times as many SNPs within R gene regions vs. intergenic regions, and there was evidence for balancing selection on R gene regions, consistent with the idea that soil pathogen pressure may be driving evolutionary dynamics of *O. bataua* R genes. Despite the fact that our targeted gene capture approach

yielded a relatively small number of R genes in a relatively small number of individuals, we found a significant difference in individual-level heterozygosity at R genes vs. intergenic regions; however, there was no detectable impact on differential progeny survival. Our results are consistent with the idea that R genes in *O. bataua* are under balancing selection to maintain genetic variation and justify further use of targeted gene capture to better understand genetic mechanisms of plant immunity in this and other non-model systems.

The importance of soil-borne pathogens in shaping patterns of plant diversity in wild systems is well established (Bagchi et al., 2014; Comita et al., 2014; Mack et al., 2019; Mangan et al., 2010), but in many cases the underlying genetic mechanisms of plant defense remain a black box. One approach to fill this knowledge gap has been to develop transcriptomes of plant species (Marden et al., 2017) or de novo genomes (Sork et al., 2022). While costs for developing reference genomes have decreased, genome size and complexity and heterozygosity may still render assembling de novo plant genomes infeasible or expensive in wild populations (F.-W. Li & Harkess, 2018) and the logistics required for working with transcriptomes can be challenging for field based studies in the tropics. *Oenocarpus bataua* and *E. guineensis* diverged ~65 mya and are in different tribes within the palm family (Baker & Couvreur, 2013), and the successful detection of R gene regions found in *E. guineensis* in our study species is consistent with findings that R gene structure and function is highly conserved in plants (Karasov et al., 2014), including palms (Puch-Hau et al., 2016). This high degree of R gene conservation over millions of years of divergence suggests that the targeted gene capture approach we employed may also work in a wide range of other non-model species. As such, we offer that the use of custom probes to sequence and study R gene regions may provide a viable alternative for studying the mechanistic

role that R genes may play in shaping observed patterns of plant-pathogen interactions in palms, and more broadly.

Our finding that the majority of R gene regions we studied have an excess of intermediate- or high-frequency alleles (Tajima's  $D > 0$ ) is consistent with balancing selection. More specifically, this result fits with the expectation of the 'trench warfare' hypothesis where a diverse and ever-evolving assemblage of soil pathogens are under positive selection for extreme alleles to overcome balancing selection for average alleles in plant R genes that provide broad immunity without incurring fitness costs (Singh et al., 2018; Tellier & Brown, 2011). These findings are in line with those reported from a number of better-studied model systems (Bakker et al., 2006; Karasov et al., 2014; Meyers et al., 2005; Rose et al., 2004; Seeholzer et al., 2010). At the same time, the presence of R gene regions with negative values of Tajima's  $D$ , though in the minority, do potentially indicate that some regions demonstrate a parallel scenario in which constant adaptation to specialized pathogen attack and plant defense leads to positive selection pressure for specialized resistance alleles (Bergelson et al., 2001). The comparatively fewer numbers of R genes under positive selection could be explained by the fitness cost of those R genes if the specialized pathogens they detect are rare across the landscape (Tian et al., 2003). It is worth noting that *O. bataua* is a hyperabundant species, and this large population size may maintain greater R gene diversity (Marden et al., 2017; Stump et al., 2020), as has been noted in other dominant tree species, including *Quercus lobata*, *Eucalyptus*, and *Populus* (Sork et al., 2022). Additional work that better resolves different selection pressures on R genes, including sampling from species with different life histories and across different populations of the same species remains a priority.

Our finding that R gene regions were significantly more heterozygous than neutral regions is concordant with the literature from agricultural and model species (Bakker et al., 2006; Buckley et al., 2018; Rose et al., 2004) and is also consistent with balancing selection maintaining R gene allelic diversity. Yet our expectation that individuals with higher levels of R gene heterozygosity should have higher progeny survival (i.e., via putatively enhanced detection and response to a broader range of soil pathogens) was not supported. It is unlikely that variation in biotic and abiotic factors experienced by seedlings, such as rainfall (Keepers et al., 2023), temperature (Cheng et al., 2013), light levels (Hua, 2013), and edaphic factors (Springer et al., 2007), would have diffused any effect of R gene heterozygosity in this study because seedlings were inter-mixed at random, but other explanations are possible. First and foremost, our sample size of  $n = 16$  adults is small, and it is possible that, with additional sampling, the non-significant positive trend we observed could potentially reach significance with greater sampling effort. Similarly, our probe selection via targeted gene capture of R genes previously reported in a different species may have resulted in a suite of R genes for study that may be biased and that is certainly far from complete. Indeed, plant species may have hundreds to thousands of R genes (Barragan & Weigel, 2021; Shao et al., 2019; Sork et al., 2022) and the 210 R gene regions in this study are almost certainly a subset of *O. batava*'s full complement.

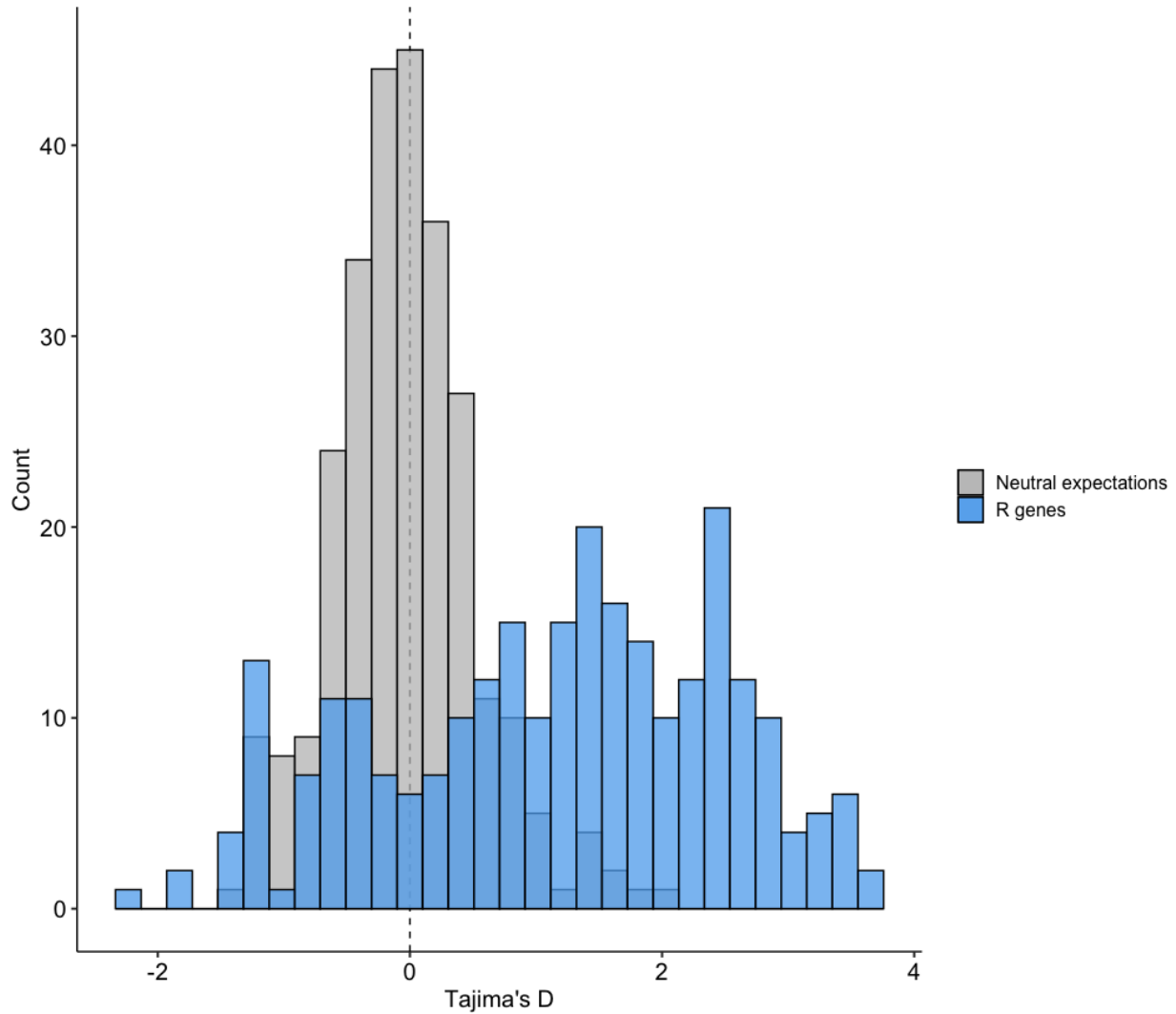
These constraints on R gene sample size represent one potential shortcoming of the targeted gene capture approach we employed. A related constraint is that this method only yields for R genes that are present and annotated in a model species (in this case *E. guineensis*), which introduces a bias for more conserved R genes. Use of a reference genome could

potentially address both these issues and improve mapping of neutral regions which were poorly represented in our study. While the targeted gene approach we employed here did provide potentially useful insights in R gene dynamics in *O. bataua*, a reference genome may avoid many of these shortcomings.

In conclusion, our target capture approach using R genes from another model species successfully detected R gene regions at a significantly higher rate than neutral regions and we detected signals of balancing selection for high rates of polymorphisms at most R genes. Our inability to find any effect of individual heterozygosity on seedling survival highlights the necessity for further genomic work to assess the ecological consequences of resistance genes in wild systems.

Individual	Probe Type	Average Heterozygosity
BR01-22859	Intergenic Regions	0.108
BR01-38200		0.109
BR01-41487		0.113
BR01-OBR-0004		0.116
BR01-OBR-0013		0.108
BR01-OBR-0062		0.111
BR01-OBR-0103		0.089
BR01-OBR-0162		0.111
BR01-OBR-0163		0.113
BR01-OBR-0175		0.101
BR01-OBR-0186		0.116
BR01-OBR-0260		0.116
BR01-OBR-0311		0.100
BR01-OBR-0356		0.126
BR01-OBR-0404		0.092
BR01-OBR-0432		0.124
BR01-22859	R gene Regions	0.335
BR01-38200		0.340
BR01-41487		0.338
BR01-OBR-0004		0.327
BR01-OBR-0013		0.345
BR01-OBR-0062		0.331
BR01-OBR-0103		0.339
BR01-OBR-0162		0.332
BR01-OBR-0163		0.326
BR01-OBR-0175		0.344
BR01-OBR-0186		0.330
BR01-OBR-0260		0.334
BR01-OBR-0311		0.349
BR01-OBR-0356		0.343
BR01-OBR-0404		0.328
BR01-OBR-0432		0.337

**SI Table 1:** Average individual heterozygosity at R gene and intergenic regions for 16 individuals of *Oenocarpus bataua*.



*Figure 1-* Histogram depicting Tajima's D statistic for R gene regions and simulated expectations. Tajima's D compares estimated nucleotide diversity of observed polymorphic sites observed against the estimated nucleotide diversity of the allele frequencies of polymorphic sites. Most R gene regions had values greater than 0, implying that these R genes are under balancing selection. Values less than 0 imply that these regions are under positive selection.

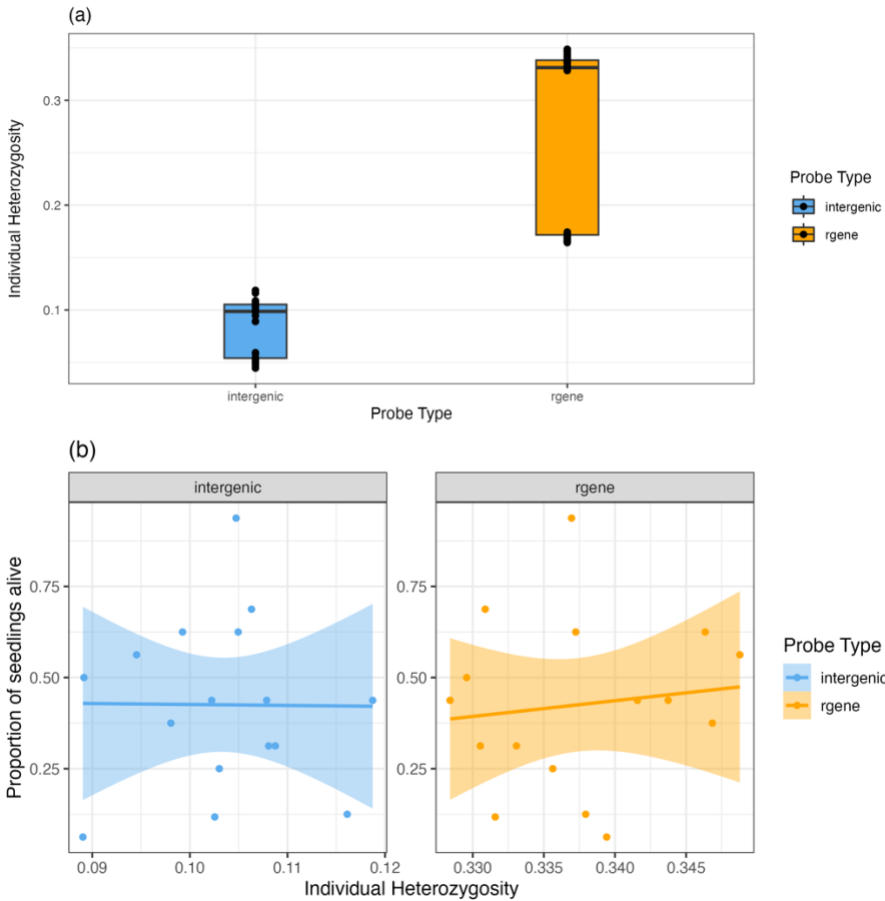


Figure 2 - (a) Box plot comparison of individual-level heterozygosity for 16 individual *Oenocarpus bataua* averaged across intergenic target probes (blue) and R gene target probes (orange). The horizontal line indicates the median value, the upper and lower whiskers show the inter-quartile range. Points show values for individual maternal trees. (b) Association between individual-level heterozygosity and proportion of progeny seedling alive in an in-field experiment for intergenic target probes (blue) and R gene target probes (orange). Each dot represents an individual maternal tree, and the line and shaded region shows a line of best fit and 95% confidence interval.

## Chapter 3: Effects of maternal identity, soil source and geographic distance on seedling growth and survival

### Introduction

Understanding the mechanisms that produce patterns of diversity is a major goal of ecologists and evolutionary biologists. One such stabilizing mechanism in forest communities, known as negative density dependence, occurs when fitness of a plant species decrease with increased frequencies of that species (Chesson, 2000; Vellend, 2010; Wright, 2002). High concentrations of natural enemies and competition with conspecific seedlings under trees lead to high mortality zones, necessitating escape to colonize favorable sites and reduce mortality rates, a concept known as the Janzen-Connell effect (Connell, 1971; Janzen, 1970). As abundance increases, successful recruitment and performance become increasingly distance and density dependent, a process described as conspecific negative density-dependence (CNDD) (Comita et al., 2010; Harms et al., 2000; Terborgh, 2012). While CNDD is widely accepted as a major force shaping tree community composition and patterns of diversity, better understanding the underlying mechanisms that generate these observed patterns is an ongoing need.

In tropical tree communities, mortality by fungal soil pathogens is thought to be an important mechanism shaping observed CNDD patterns (Bagchi et al., 2014; Comita et al., 2014; Mack et al., 2019; Mangan et al., 2010). While it is known that many soil microbes specialize on particular plant families and species (Gilbert & Webb, 2007), more recent work suggests within-species specialization (i.e., on different host genotypes) may also be an important, but underappreciated, source of seedling mortality. For example, both experimental and correlational studies indicate that seedlings of the palm *Oenocarpus bataua* with rarer genotypes experience a significant survival advantage, equivalent in effect size to access to light

(Browne & Karubian, 2016, 2018). Moreover, a shadehouse experiment with *Virola surinamensis* showed that seedlings perform better in soil from other conspecific adults than in soil from their maternal tree, also consistent with host genotype specificity by soil pathogens (Eck et al., 2019). Similarly, another shadehouse experiment found that seedling survival rates of two subtropical tree species increased with geographic distance and genetic dissimilarity between populations of conspecifics (X. Liu et al., 2015). While few in number, these studies suggest that, just as host specialization by pathogens at the between-species level plays an important role in CNDD, pathogens focused on specific host genotypes may also drive differential survival among individuals within a single plant species (Eck et al., 2019). If true, this within-species specialization by soil pathogens would have significant implications for our understanding of the processes that shape diversity in these systems, by introducing a currently under-appreciated determinant of seedling performance that may interact with CNDD and other forces to shape observed patterns of diversity.

If within-species specialization of soil pathogens occurs, then we would expect to find pathogenic strains specialized on a particular individual tree's genotype clustered around that individual tree, such that the impact of soil pathogens beneath adult trees would diminish as the geographic distance between two conspecific trees increases. Eck et al. (2019) did not find support for this expectation in *V. surinamensis*, but to our knowledge this expectation has not been experimentally examined in other systems. More broadly, patterns and relevant spatial scales of soil pathogen community composition decay are not well understood in tropical environments (Green et al. 2004). Distance-decay has been noted in bacteria (Cho & Tiedje, 2000), fungi (Branco et al., 2013; Green et al., 2004; Shi et al., 2014) and other eukaryotic soil

microbes (Hillebrand et al., 2001) and the effect of geographic distance on fungal community similarity may vary with the degree of host specialization and niche (i.e., mutualist vs. pathogen) (Chaithaisong et al., 2022), but the geographic scale at which these effects occur remains poorly understood. Tropical, pathogenic, root-associated fungal community composition has been found to vary with increasing distance up to 30m, which was largely explained by phylogenetic relatedness of the host species (Schroeder et al., 2019). Quantifying the effect of geographic distance, and its potential interactions with host relatedness, may help to improve our understanding of density dependent mortality on seedlings and, by extension, community structure.

In the current study, we explore the degree to which soil collected from beneath different individual conspecific adult trees of the canopy palm species *Iriartea deltoidea* influences seedling survival and performance. Using a shadehouse experiment to assess survival and growth of seedlings collected from a single continuous population, we set out to answer two fundamental questions. First, can we detect a difference in seedling survival and performance when they are planted in soil collected from beneath their own mother ('maternal soil') vs. soil collected from another conspecific adult from the same population ('heteroconspecific soil')? Second, can we detect any impact of the geographic distance separating pairs of conspecific adults on seedling survival and performance? We hypothesized that seedlings planted in soil gathered from adults at closer geographic distances to the maternal source would exhibit decreased survival and performance, due to two underlying but not directly tested assumptions: (1) community turnover of soil microbial communities will increase with distance and (2) genetic differences between conspecific trees, which in turn may

shape the composition of pathogenic microbial communities in the soil beneath those trees, will also increase with distance.

## Methods

### *Study System and Focal Species*

The study was conducted in the reserve of Fundación para la Conservación de los Andes Tropicales (hereafter FCAT; 79°39.91'W 0°22.38'N: 184-549m elevation, 2-3 m precipitation annually). The FCAT Reserve contains ~750ha of primary and secondary humid rainforest within and around the Mache-Chindul Reserve in the Chocó biogeographic region of Northwestern Ecuador. The Chocó biogeographic region is highly threatened and boasts exceptional plant and animal biodiversity and endemism (Myers et al., 2000).

*Iriartea deltoidea* (Ruiz & Pav.; Arecaceae) is a canopy palm widely distributed in wet lowland and premontane forests of western Amazonia, the Tumbes-Chocó, and Central American region (Álvarez-Loayza et al., 2011). It is considered a hyper-abundant species and is one of the most common trees in Amazonia (Ter Steege et al., 2013) and in our project area (J. Karubian, P. Fine and M. Silman, unpublished data). *Iriartea deltoidea* is monoecious and insect-pollinated and produces single-seeded fruits in large infructescences of ~1,000 – 2,000 fruits, serving as a keystone resource for multiple frugivorous animals (Henderson, 2002).

Additionally, *I. deltoidea* is culturally and economically important to local communities (Fadiman, 2019) and is commonly left as a remnant tree in cleared pastures in our project area.

### *Seedling Survival and Performance Experiment*

Nursery soil preparation and sterilization

Soil used in the seedling experiment was sourced from topsoil gathered from a site ~30 km away from FCAT (79°30.88'W 0°16.91'N, 301m asl) containing qualitatively similar soil. After transporting the soil to the FCAT station, we sieved the soil in a 2 mm mesh to remove rocks and debris. The soil was then treated by a hand mister with a combination of two fungicides used in previous studies to target pathogenic fungi and oomycetes (Bagchi et al., 2014; Krishnadas et al., 2018): Amistar (Syngenta Ltd) and Ridomil Gold (Syngenta Ltd) following manufacturer's guidelines (Amistar: 0.01 g, Ridomil: 0.5 g, each dissolved in 100 ml water). Following application of these fungicides, the soil was placed between two 0.2 mm thick low-density polyethylene (LDPE) sheets in direct sunlight to heat the soil using captured irradiation for 4 weeks (Kanaan et al., 2018). Volatile chemicals released during heating have been noted to significantly reduce richness and diversity of non-thermophilic soil-borne fungal and bacterial communities (Gamliel et al., 2000; Kanaan et al., 2016; Simmons et al., 2014).

#### *Seed collection, preparation, and germination*

Within the FCAT reserve, we identified and monitored the phenology of 21 adults of *I. deltoidea* with maturing fruits close to being ripe (as judged by fruit coloration) and mapped the location of each tree with a handheld GPS. We then harvested approximately 200 mature fruits directly from the infructescence from 15 of these adults in a two-day period (26-27 Jan 2021), allowing us to synchronize the developmental stage of all seedling cohorts used in this experiment. Each cohort of fruits collected from each adult was separately stored in open plastic bins in a cool, dark location and covered with mesh to exclude insects. Electric fans were used to maintain air circulation during storage.

Fruits were stored for 10 days until processing. All seeds were prepared for planting in a two-day period (5-6 February 2021) using the following methods. The seeds were soaked in water and the epicarp and mesocarp were carefully removed with pliers. We next sterilized the seed surface by soaking each seed in 10% bleach for 5 minutes, followed by 5 minutes in 20% ethanol (Sauer & Burroughs, 1986). Seeds were then placed with even spacing within bins under netting and allowed to air-dry, with air circulation provided by an electric fan. Seeds were then visually inspected and excluded if signs of damage or decomposition were present. 150 seeds from each of the 15 adult trees were then planted in batches in 10 cm of sterilized soil in plastic bins (60 cm x 40 cm x 30 cm) at a depth of 2 cm. We ensured the seeds were well-watered and allowed to germinate and grow for 12 weeks, the minimum time at least 75% of seeds in each batch had germinated.

#### *Reciprocal planting*

We filled each of 2250, 2-l, 0.09mm thick, low-density polyethylene plastic bags designed for growing seedlings with the 3:1 sterile soil-sand mixture described above. To obtain soil inoculum for our experiment, we collected and homogenized soil from beneath each of the 15 adults by sampling three points located 2 m from the trunk at a depth of 10 cm. To avoid contamination, soil was placed into a sterilized bucket with an unused, sterilized trowel and sealed with plastic wrap. This sampling was conducted from 25-26 May 2021, 1-2 days before the germinated seedlings were each planted in a separate plastic bag on 27 May 2021. The homogenized soil from each adult was sieved in individual batches to remove rocks, roots, and other debris with a previously unused, sterilized sieve. Into each bag, we added a 120 g plug (6% total soil volume) of live soil inoculum from one of the 15 adults and then planted a single,

seedling one month after the minimum germination time. Differences in seedling size were accounted for in the experimental design (see below).

Seedlings were planted into bags containing either their maternal tree's live inoculum (maternal soil) or inoculum from each of the 14 heteroconspecific source trees (heteroconspecific combinations), with ten replicates for each of these 15 treatments. As such, for the 150 seedlings used in this experiment from each of the 15 source trees, 10 seedlings were individually planted with maternal inoculum and 10 seedlings were individually planted in bags with each of the 14 possible heteroconspecific combinations in a full-factorial design. This design leads to 15 source trees \* 150 seedlings per source tree, totaling 2250 seedlings. Each of the 2250 experimental seedlings was individually marked and randomly placed within one of 10 blocks in the shadehouse, with each block containing 225 seedlings, corresponding to one full replicate of the full factorial design with every possible combination of source tree seedlings and soils. This design was employed to account for any potential differences associated with the initial size of progeny from source trees at the onset of the experiment, or with growing conditions in different locations within the shadehouse. To mimic shady understory conditions, the shadehouse roof was constructed of clear 0.2 mm thick low-density polyethylene (LDPE) sheets lined with two layers of high-density polyethylene netting to simulate 80% shade cloth.

Seedlings were visually monitored daily and watered regularly, with surviving seedlings collected on 29 November 2021, six months after planting in the shadehouse. After harvest, seedlings were measured for stem height, stem diameter, wet above-ground biomass, wet below-ground biomass, and leaf area. Leaf area was measured for the largest leaf by scanning the leaves and processing in ImageJ (Schneider et al., 2012). After measurement, seedlings

were placed in paper bags and dried in an oven at 70 degrees F for 3-4 days. After drying, we recorded dry above and below-ground biomass using a scale to the nearest 0.01 (SPX422, Ohaus Corporation, Parsippany, NJ, USA). Some plant tissue was damaged in the drying process, and we were unable to measure dry weights in 183 samples. As tissue was dried in block order, the missing data were evenly distributed across all source trees and soil combinations.

### *Statistical Analyses*

To test the effect of maternal source, soil source, and geographic distance on seedling survival, specifically whether a seedling survived to harvest or not, we fit a binomial general linear mixed effects model using the *lme4* package (Bates et al., 2015) in R 4.2.1 (R Core Team, 2022). Seedling survival was binomially encoded as the response variable, with maternal source, soil source, and scaled distance between maternal and soil plug source as the predictor variables. The block number was included as a random effect. We calculated pairwise geographic distance between maternal source and soil plug source for each individual seedling in the experiment using the *geosphere* package (Robert J. Hijmans, 2022) in R 4.2.1. Pairwise distance was modeled both as a linear and quadratic terms in separate models, but as they were quantitatively similar, we report only the model with linear pairwise distance. All effect sizes were calculated using the *effectsize* package (Ben-Shachar et al., 2020) in R 4.2.1.

Only seedlings that survived to harvest were measured (above) and used in subsequent statistical analyses to evaluate performance. As stem height, stem diameter, wet and dry above-ground biomass, wet and dry below-ground biomass, and leaf area were highly correlated, we performed Principal Components Analysis (PCA) to consolidate the variables using the base *stats* package (R Core Team, 2022) in R 4.2.1. Variables used in the PCA were

centered and scaled. We initially ran a total of three PCAs. The first PCA used only seedlings grown in sample blocks in which all seedlings were measured (N=1013 seedlings), excluding entire blocks if one or more seedlings in the block was damaged in the drying process. The second PCA included all blocks but excluded seedlings with missing dry biomass measurements (N=1087). The third PCA included all harvested seedlings (N=1270) but excluded dry biomass from the analysis. As the results for all three PCAs were qualitatively similar, we used the coefficients in the first PCA as response variables in separate models.

We next fitted a series of linear mixed effect models with orthogonal contrasts using the interaction between seedling maternal source and soil source, as well as pairwise geographic distance as predictor variables with block number as a random effect. We used the first and second principal components from the first PCA as the response variables, respectively.

To test the effect on performance of seedlings planted in maternal soil vs. soil of other heteroconspecifics, we fitted a linear mixed effect model with total biomass as the response variable. The predictor variables were a categorical variable of whether the seed was planted in maternal soil or not with block, maternal source, and soil plug source as random variables.

## **Results**

### *Adult selection and seedling survival*

We used 15 adults located within FCAT (Fig. 1A) as the source trees for this experiment; mean pairwise distance between these adults was 1443.19m (min=131.72m, max=2836.12m, sd= 804.86) and mean elevation was 464.47m (min=405m, max=586m, sd= 44.26). Of the 2250

germinated seedlings that were transplanted for the full experimental design, 1270 (56.4%) survived until harvesting and measurement.

### *Seedling survival*

We hypothesized that seedling survival should vary with the geographic distance between the maternal source tree and the soil plug source, but not with maternal identity or soil plug source *per se*. However, maternal source, soil plug source, and geographic distance all significantly influenced seedling survival (Table 1), with the effect of maternal identity ( $\omega = 0.36$ , 95% CI [0.32, 0.40]) being substantially stronger than that of soil plug source ( $\omega = 0.12$ , 95% CI [0.08, 0.16]) or geographic distance ( $\omega = 0.04$ , 95% CI [0.00, 0.08]) (Fig. 2C). Notably, we observed a three-fold difference in mean progeny survival rates of different maternal source trees, from a maximum of 86.7% (sd= 0.105) to a minimum of 26.4% (sd=0.13) (Fig. 2A, SI Table 1).

### *Seedling performance*

The first and second principal components used to characterize seedling performance explained 82.1% of variance (sd= 2.03) and 8.5% of variance (sd= 2.23), respectively. PC1 was related to the majority of the measurements we took, and we use it as a composite index of seedling performance, while PC2 differentiated between above-ground and below-ground soil metrics (Table 2).

PC1, our composite index of seedling performance, was strongly influenced by both maternal identity of the seed ( $\omega = 0.47$ , 95% CI [0.43, 0.52]) and the interaction between the maternal identity and soil plug source ( $\omega = 0.37$ , 95% CI [0.33, 0.41]) (Table 3, SI Fig. 1). Soil plug

source had a weaker but significant effect ( $\omega=0.13$ , 95% CI [0.09, 0.17]) on composite seedling performance. We found no evidence that geographic distance between maternal source tree and soil plug source influenced seedling performance. Only maternal source tree identity strongly influenced ( $\omega =0.35$ , 95% CI [0.31, 0.39]) above vs. below-ground performance, as measured by PC2.

#### *Effect of maternal vs heteroconspecific soil*

We found no evidence that being planted in maternal vs. heteroconspecific soil influenced seedling survival or performance (Table 4, Fig.4).

### **Discussion**

In this study, we examined the effect of maternal tree identity, the soil plug source, and the geographic distance between maternal source tree and the soil plug source on survival and performance of seedlings of *Iriartea deltoidea*, a Neotropical canopy palm. We also examined the effect of being planted in maternal vs. heteroconspecific soil on survival and performance. Our aim was to investigate whether introducing seedlings to soil microbial communities beneath different conspecific trees might influence seedling performance and survival, and if so, the ways in which this relationship might vary with geographic distance between adults. We found that maternal identity, soil plug source, and geographic distance all significantly influenced seedling survival, with maternal identity having the largest effect by a wide margin. When evaluating overall seedling performance, we found that that maternal identity, soil plug source, and their interaction influenced overall performance, with maternal identity again

having the strongest effect. Lastly, we found no relationship between seedling survival or seedling performance when planted in maternal vs. heteroconspecific soil. Our results point to strong differences in overall progeny survival and performance among different adults in our study population and suggest that finer-grained relationships with soil and geographic distance merit further work.

Our study revealed significant impacts of maternal identity on both seedling survival and performance, with a three-fold difference in mean progeny survival rates between the highest and lowest performing maternal trees. Substantial variation in mean progeny performance between maternal sources was observed by Eck et al. (2019) in *V. surinamensis*, but the difference between highest and lowest performing maternal trees in that study was approximately half that seen in our study. Genotypic diversity and heterozygosity in maternal trees have been linked to differences in progeny competitive abilities (Cahill Jr et al., 2005), resistance against herbivore predation (Wise, 2007), and susceptibility to pathogens (Marden et al., 2017; Stump et al., 2020), and could help to explain these results. It is also possible that genetic dissimilarity of a given adult relative to the broader population might result in enhanced seedling survival and performance, by providing escape from soil pathogens that are focused on more common genotypes in the population. For example, in *O. bataua*, another prevalent canopy palm species, maternal trees with rare genotypes in our project area experienced higher rates of seedling survival (Browne & Karubian, 2018). Independent of other factors we evaluated, we also found a strong impact of different soil plugs on mean seedling survival and performance, highlighting the need to characterize these soil communities to better understand the relative impact of different types of soil microbes and strains. More

broadly, using genomic analyses to explore relationships between tree genotypes, soil microbial communities, and seedling survival and performance would provide a deeper understanding of the mechanisms underlying such striking variation in survival and performance of the maternal trees used in this study.

We also found that increasing geographic distance between a seedling's maternal identity and its soil plug source significantly increased seedling survival, although the effect was not as strong as that of maternal tree identity and did not impact seedling performance. This distance-dependent effect on seedling survival is consistent with Janzen-Connell mechanisms, in which escape from specialist predators near the maternal tree (in this case, soil pathogens specialized on the maternal tree genotype) increases seedling survival. In this scenario, decay in pathogen community similarity with increasing geographic distance between trees, increasing genetic differentiation between trees with increasing geographic distance, or some interaction between the two, may provide survival advantages. Pairwise distances between neighbors among the maternal trees used in this study ranged from ~132 to 2836m (although it is important to note that *I. deltoidea* is an abundant species on our study site and other adults were also present but not included in this study), and previous work has found that fungal community similarity decays quickly over distances < 50m (Kivlin & Hawkes, 2020; Schroeder et al., 2019). However, processes underlying this relationship with geographic distance remain speculative at this point, and other explanations are also possible. For example, genetically similar seedlings may tend to utilize and thus compete for similar resources, resulting in higher density-dependent mortality under maternal trees, although we attempted to mitigate this effect by planting all seedlings in homogenized, sterilized soil collected from a single source.

Additional work assessing the genetics of adult trees and soil pathogen communities are required to better understand these potential relationships.

Previous studies (Eck et al., 2019; Wang et al., 2022) found that parental trees exerted a strong negative effect on offspring and this negative effect weakens when planted in soil from increasingly spatially distant adults. In our study, both survival and performance of seedlings weakly decreased when planted in maternal soil, but this effect was not statistically significant. However, we did find a significant interaction between soil plug source and maternal tree identity, meaning that progeny from certain maternal trees consistently performed better (or worse) in soil collected from beneath particular heteroconspecifics. A follow up study evaluating whether variation in seedling performance is correlated with genetic distance could provide further information on whether soil pathogens adapted to attack certain genotypes within a species may be influencing this pattern; in this case, we would expect that increased genetic distance between adults and associated soil microbial communities would be associated with increased seedling performance.

There are several caveats to consider in the current study. Our use of fungicides and insolation may have been insufficient to equally sterilize soil of all beneficial and pathogenic microbes. Future work using autoclaved soil would be desirable, as would genetic characterization of the soil microbe communities beneath maternal trees and in our experimental seedling bags to better understand the environments experimental seedlings were subjected to. It is also worth noting that *I. deltoidea* is hyperabundant, meaning that many other adult individuals were present on our study site but not included in our study, and

also that different patterns might be observed in less abundant tree species. Finally, it would be useful to evaluate whether these patterns are present at different spatial scales.

In summary, while our results are consistent with within-species specialization by soil pathogens, they highlight the need for additional work to understand genetic mechanisms, impacts of different components of the soil microbial community, and finer-grained relationships with geographic distance. In particular, quantifying the degree of genetic dissimilarity between trees, and between soil microbial communities associated with those trees in a spatially explicit format and relating that information to seedling survival and performance is a clear priority. Future work should also incorporate different tree species across a spectrum of abundances and life history traits to fully assess the importance of maternal identity. Together, these will likely provide important insight into the role that intraspecific negative density dependent mortality by soil pathogens plays in the maintenance of plant diversity.

<b>Term</b>	<b>Statistic</b>	<b>df</b>	<b><math>\omega</math></b>	<b>P value</b>
Intercept	2.219	1	0.02	0.136
Mother	298.069	14	0.47	<b>&lt;0.001</b>
Soil	33.204	14	0.13	<b>&lt;0.001</b>
Geographic Distance	4.312	1	0.04	<b>0.038</b>

**Table 1: Model coefficients from the binomial general linear mixed effects model for *Iriartea deltoidea* seedling survival rates in full factorial shadehouse experiment.** Predictors in this model included the maternal source of a seedling (Mother), the soil plug source a seedling was planted into (Soil) and the scaled geographic distance between the between maternal and soil plug source. The experimental block number was included as a random effect. All three predictors were significant. Seedling survival was positively associated with geographic distance, and maternal source of a seedling most strongly explained survival by a wide margin.

	<b>PC1</b>	<b>PC2</b>
Standard deviation	4.103	0.425
Proportion of Variance	82.07	8.490
Stem Diameter	-0.454	0.22
Stem Height	-0.449	0.119
Below-ground wet weight	-0.397	-0.9109
Above-ground wet weight	-0.481	0.165
Leaf Area	-0.451	0.286

**Table 2: PCA eigenvectors (standard deviation), variance explained, and loadings for the top two principal components from PCA conducted on performance metrics gathered from harvested seedlings.** PC1 and PC2 together explained 90.56% of variation. We interpreted PC1 as overall seedling performance and PC2 as the above vs. below ground performance.

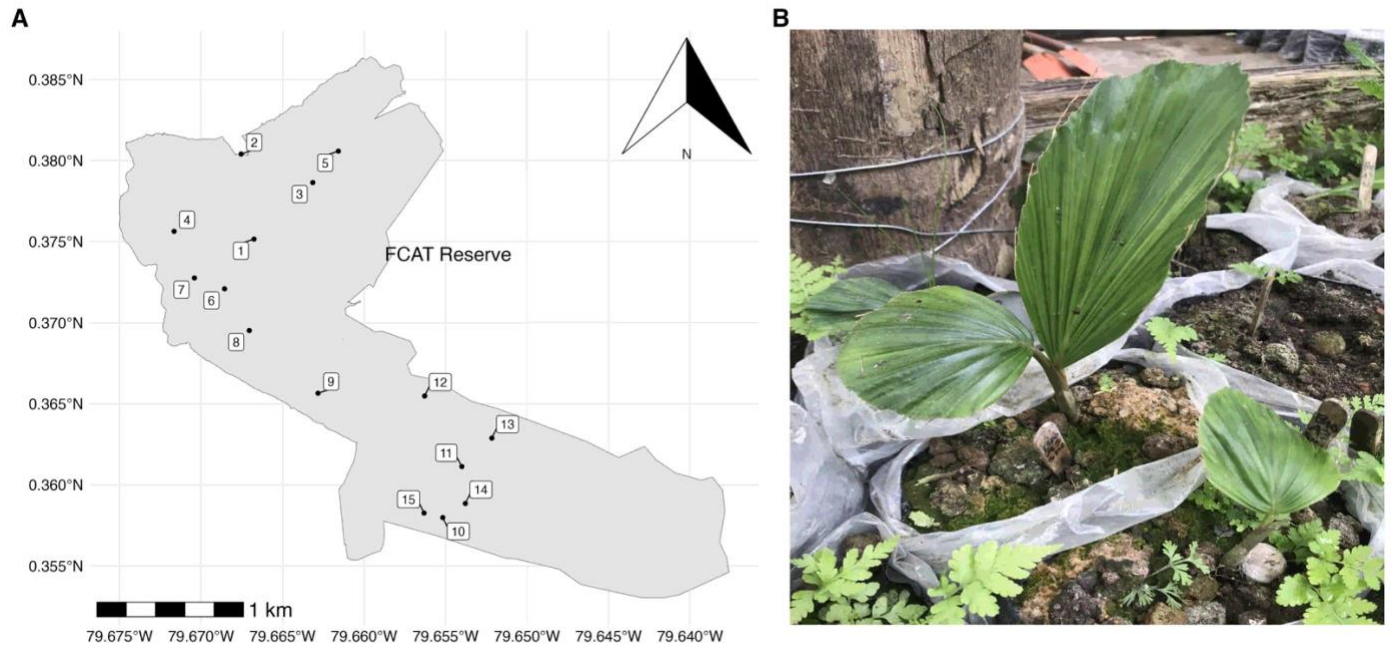
Response	Term	Statistic	df	$\omega$	P
Overall Performance (PC1)	Intercept	17.482	1	0.09	0.000
	Mother	507.585	14	.047	<0.001
	Soil	39.113	14	0.13	<0.001
	Geographic Distance	0.136	1	0.00	0.712
	Mother:Soil	309.012	195	0.37	<0.001
	Above vs. Below-ground Performance (PC2)	Intercept	7.091	1	0.05
Above vs. Below-ground Performance (PC2)	Mother	274.346	14	0.35	<0.001
	Soil	6.398	14	0.05	0.955
	Geographic Distance	0.021	1	0.00	0.884
	Mother:Soil	211.580	195	0.00	0.198

**Table 3: Model coefficients from linear mixed effect models with orthogonal contrasts of**

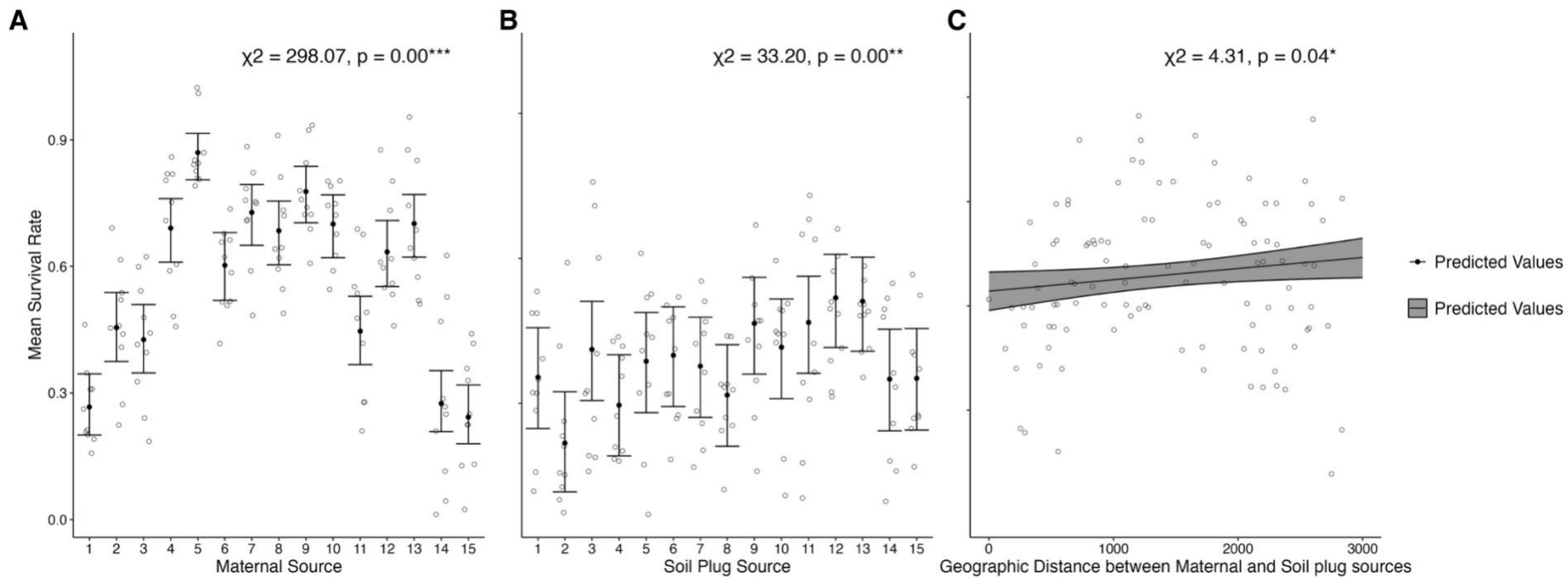
**seedling performance of *Iriartea deltoidea*.** Predictor variables for all models included the maternal source of a seedling (Mother), the soil plug source a seedling was planted into (Soil), the scaled geographic distance between the between maternal and soil plug source, and the interaction between seedling maternal source and soil plug source. Block number was included as a random effect. Maternal source, soil plug source, and their interaction were significant for the overall performance model, while maternal source alone was significant for the above vs. below-ground performance model. In all models, maternal source had the largest effect on model outcomes.

<b>Response</b>	<b>Term</b>	<b>Statistic</b>	<b>df</b>	<b><math>\omega</math></b>	<b>P value</b>
General Performance (PC1)	Intercept	68.357	1	0.17	0.000
	MS	0.509	1	0.00	0.475
Above vs. Below-ground Performance (PC2)	Intercept	42.823	1	0.14	0.000
	MS	1.846	1	0.02	0.174
Mean Survival	Intercept	0.082	1	0.00	0.775
	MS	1.499	1	0.01	0.221

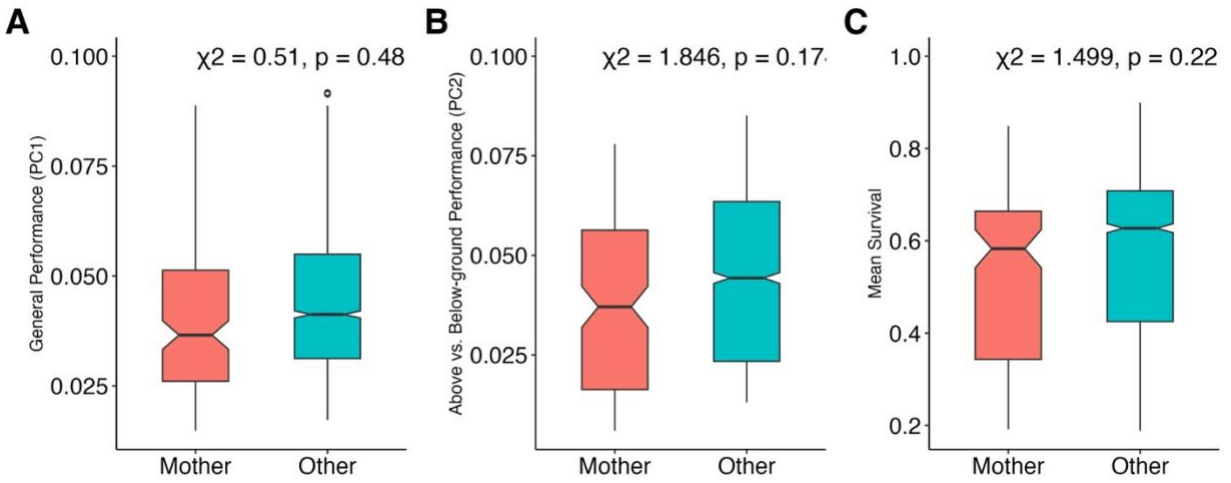
**Table 4: Model coefficients from linear mixed effect model with total biomass of *Iriartea deltoidea* seedlings as the response variable to test the effect of being planted in maternal source vs. soil from another adult.** The predictor was a categorical variable of whether the seedling was planted in maternal soil or not (MS). Experimental block, maternal source of a seedling source, and soil plug source were included as random variables random variables. No significant relationships were detected.



**Figure 1:** (A) Map of FCAT reserve in northwest Ecuador, with locations of adult *Iriartea deltoidea* used in this experiment, and (B) an *I. deltoidea* seedling at ~10 months of age, immediately prior to harvesting.



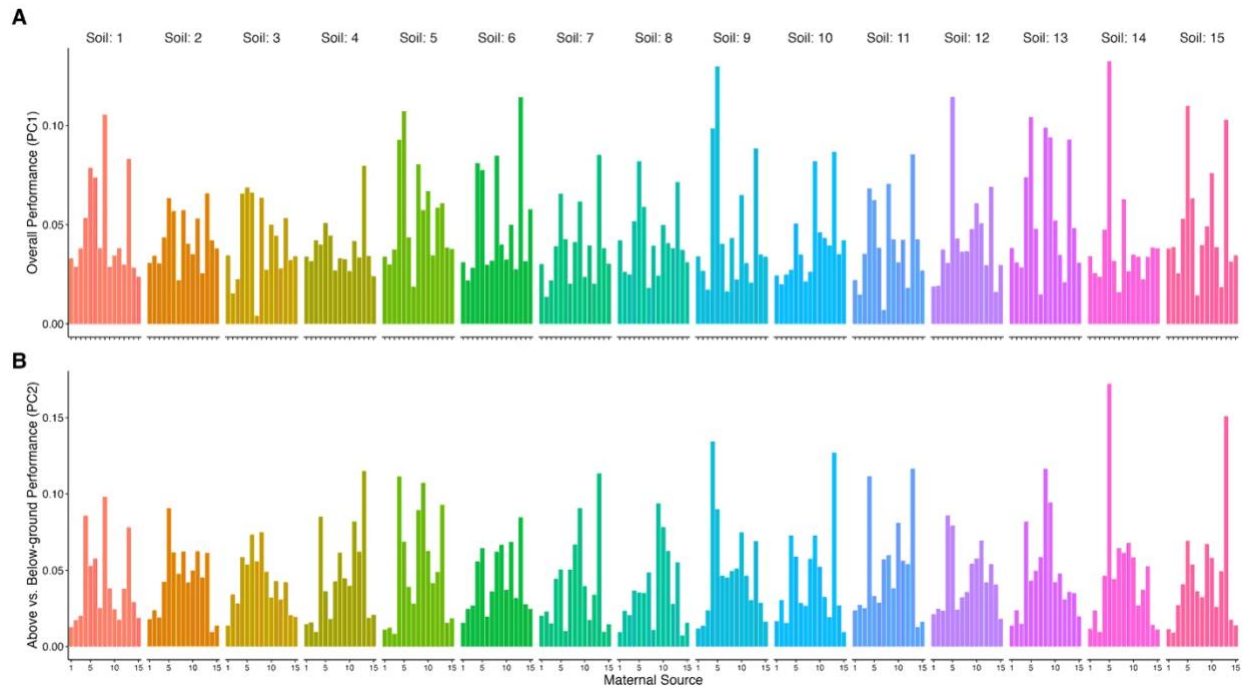
**Figure 2:** The relationship between predicted mean survival rate of seedlings and (A) a seedling’s maternal identity, (B) soil plug source tree, and (C) the geographic distance between maternal source tree and soil plug source. Seedling survival varied significantly with all three, with maternal source having a considerably stronger effect size. Error bars denote standard error and significant comparisons are denoted by an asterisk (\* $P < 0.01$ ).



**Figure 4:** The effect of a seedling planted in soil gathered from its maternal tree vs. a heteroconspecific on **(A)** general performance, as measured by PC1; **(B)** above vs. below-ground performance, as measured by PC2; and **(C)** survival. No significance was detected.

<b>Mother</b>	<b>Mean Survival Rate (<math>\pm</math>SD)</b>
1	0.29 $\pm$ 0.15
2	0.46 $\pm$ 0.21
3	0.46 $\pm$ 0.16
4	0.69 $\pm$ 0.16
5	0.87 $\pm$ 0.1
6	0.59 $\pm$ 0.22
7	0.72 $\pm$ 0.2
8	0.67 $\pm$ 0.21
9	0.77 $\pm$ 0.16
10	0.7 $\pm$ 0.22
11	0.45 $\pm$ 0.22
12	0.63 $\pm$ 0.18
13	0.7 $\pm$ 0.12
14	0.3 $\pm$ 0.16
15	0.26 $\pm$ 0.13

**SI table 1:** Mean survival rates of seedlings by their maternal source. The maternal source with the highest mean survival was approximately three times larger than the lowest surviving maternal source.



**SI Figure 1.** The relationship between **(A)** predicted overall seedling performance and **(B)** above vs. below ground performance with seedling maternal identity and soil plug source tree. Maternal identity, soil plug source and their interaction all significantly explained overall performance, while maternal identity alone significantly explained above vs. below ground performance.

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