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**Determining Impediments to Gene Flow in a Natural Population of *Cornus florida* L.
Cornaceae, Using Integrative Landscape Genetic Techniques**

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science,
at Virginia Commonwealth University.

By
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Master of Science
Virginia Commonwealth University
2011

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Abstract

DETERMINING IMPEDIMENTS TO GENE FLOW IN A NATURAL POPULATION OF *CORNUS FLORIDA* L. CORNACEAE, USING INTEGRATIVE LANDSCAPE GENETIC TECHNIQUES

By Crystal A. Meadows, M.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2011

Major Director: Dr. Rodney J. Dyer, Associate Professor, Department of Biology

This study examined the impact intervening environment has on gene flow in the insect pollinated understory tree, *Cornus florida* L., by combining GIS and landscape genetic techniques (Least Cost Path Analysis, Circuit Theory, and Conditional Genetic Distance). Traditional population genetic analysis indicated pair-wise relatedness was significantly correlated to distance (Pearson; $r = -0.312$, $P < 0.001$) suggesting a spatial component to offspring relatedness. Dispersal throughout the study site was non-random, exhibiting a high degree of pollen pool structure due to restricted gene flow (Two-Generation Analysis; $\Phi_{ft} = 0.161$, $P = 0.001$). Forest structure was quantified in GIS layers representing coniferous canopies, mixed hardwood canopies, *C. florida* canopies, open understory (roads), and open understory/canopy due to tree removal. Of these layers, landscape isolation for the roads layer provided the best-fit model for describing genetic differentiation among sampled pollen pools

(Mantel; $r = 0.542$, $P = 0.001$). These data also suggest that improved biological inferences can be gained by examining a range of landscape isolation models.

Introduction

Gene flow is the movement of either gametes or individuals among populations resulting in the exchange of genetic material (Devlin & Ellstrand, 1990; Hedrick, 2005). When the rate of gene flow is high, mixing of genetic material among populations leads to homogenization of genetic structure. This can increase effective population size (N_e), weaken genetic drift (e.g. the stochastic loss of diversity), and disperse and establish foreign genes into the population gene pool (Devlin & Ellstrand, 1990; Schnabel & Hamrick, 1995; Hedrick, 2005). Conversely, when gene flow is low, populations can become genetically differentiated via mechanisms such as genetic drift, mutation, and selection (Hedrick, 2005). Independent of the rate of movement, an almost universally overlooked component of gene flow is the extent to which heterogeneity in the environment through which genes are dispersed may have differential influence on dispersal.

In plants, pollen-mediated gene flow, the most spatially pervasive vector of gamete movement, provides important insights into contemporary population dynamics and may also shed light on the extent to which the physical and environmental influences gene movement (Burczyk & Koralewski, 2005). There are two general modes of pollen dispersal: abiotic dispersal such as wind/gravity (conifers, coconuts, and several temperate forest trees) and water (largely restricted to aquatic angiosperms) and biotic dispersal facilitated by animals (mostly insects though several plant taxa also use vertebrates). For wind-mediated pollen dispersal, large amounts of pollen are either actively or passively released into the air column and wind convection serves as the vector of distribution (Okubo & Levin, 1989). Intervening landscape

features that may retard successful wind pollination may include physical distance (even pollen has a settling velocity), physical barriers such as mountains and valleys, and unreceptive maternal trees. For insect mediated movement, the mechanism and patterns of dispersal are more complex and to a large extent are thought to be influenced by site-specific ecological and community conditions. For example, Dick *et al.* (2003) showed that in *Dinizia excelsia*, a tropical rainforest tree typically pollinated by stingless bees, primary pollinators switched from native species to Africanized honeybees when the landscape was fragmented due to agriculture. This fragmentation-mediated switch facilitated a change in not only the species that were providing pollination services, but also resulting pollination dynamics of the population at large. Resulting population genetic structure for the tree was also impacted because Africanized honeybees generally travel longer distances than the native stingless bee species (Dick *et al.*, 2003).

Even if local conditions do not change, pollinator behavior can also be impacted by site-specific biotic and abiotic factors (Brunet, 2005). For example, wind throws and resulting gaps created by storms can influence local microclimates. Consequently, abiotic factors such as temperature (especially surface temperatures), and light can change between open and closed canopy regions within a single forest stand. Stands without canopy breaks will tend to have higher humidity, a more constant temperature and reduced wind than open canopies created by wind throws (Runkle, 1985). Disturbances in the canopy structure can also affect insect population densities, which can influence plant-pollinator interactions at the community level (Schowalter, 1985, Carr 2010). For example, Gardiakos (2011) found that consanguineous mating (s_b , mating among relatives) and correlated paternity (r_p , the probability that different offspring in the same maternal individual will be sired by the same father) in flowering dogwood

(*Cornus florida* L.) were significantly higher in sites with open primary canopies than in stands with closed canopies ($s_{b \text{ open}} = 0.162$, $s_{b \text{ closed}} = 0.112$, and $r_{p \text{ open}} = 0.212$, $r_{p \text{ closed}} = 0.063$). She argued that these results are due to non-random pollen sampling caused by restricted pollinator movement and limited pollen sources. Reese and Barrows (1980) studied *Claytonia virginica* and its pollinator *Andrena erigeniae* (a taxon that also pollinates *C. florida*) and found that these insects were attracted by sunlight and density of flowers when pollinating. Herrera (1995) also found that the insect-pollinated shrub *Lavandula latifolia* had pollinators that preferred high irradiance sites to other sites with lower irradiance showing support that the microclimate surrounding trees also influences pollination.

While canopy structure can have direct influences on pollinator communities, it is becoming increasingly clear that these differences also have indirect effects on genetic structure of offspring. Sork *et al.* (2005) showed that *C. florida* silvicultural practices can influence patterns of gene flow sufficiently that the genetic structure of offspring pools are significantly different. In that study, *C. florida* individuals were sampled from uncut, selective cut, and clear-cut forest plots in the Ozark mountains of southern Missouri. Their findings show lower pollen pool structure (e.g., a more homogenous pollen donor population) in clear-cut stands ($\Phi = 0.09$) than in uncut stands ($\Phi = 0.174$) with selective cut stands falling in the middle ($\Phi = 0.125$). These numbers indicate that canopy dynamics play a role in the movement of pollen through forest understories.

There are many approaches that are used to quantify spatial patterns of pollen-mediated gene flow. Perhaps the most common approach is that of paternity analysis. Here all adult trees in the population (or within a pre-defined area within the population) are genotyped for several loci. A subset of mothers are chosen from within that region that provide large seed arrays (at

least half-siblings at the level of the mother tree). Seeds are germinated along with the maternal individual, and the genetic profile of the pollen donor for each seed is estimated by subtracting the maternal contribution to each of the offspring's diploid genotypes. This method is quite labor intensive, and it can be hard to locate every possible male contributor in a population (Dow & Ashley, 1998, Devlin & Ellstrand, 1990). Moreover, there are restrictions on the statistical power of inferences gained if the number of seeds collected per mother does not exceed the number of potential fathers in the population. As a result, it is common to see relatively small spatial areas under investigation, which limits the applicability of the findings to larger spatial and temporal scales.

Indirect approaches, that do not quantify all the potential pollen donors, have also been used to examine pollen-mediated gene flow. At the most restricted spatial scale, mating-system analysis (Ritland, 2002) examines offspring from a set of maternal trees and attempts to determine selfing and inbreeding rates. Specific mating-system parameters include the multilocus selfing rate (s_m , perhaps the most restricted dispersal of pollen), the rate of biparental inbreeding/consanguineous mating (s_b , mating amongst relatives), and correlation of paternity (r_p , the likelihood that two offspring on a maternal tree are actually full sibs). Increases in selfing rates can occur when there are few father contributors surrounding an individual mother tree (e.g., outcross pollen limitation; Farris & Mitton, 1984). Biparental inbreeding (s_b) can indicate if trees within the population are more likely to mate with closely related individuals (Ritland, 2002), which in most tree populations that exhibit positive spatial autocorrelation, translates into nearest (or close) neighbor mating. With *C. florida*, the species studied in this thesis, realized seed dispersal was measured to extend beyond the canopy of the maternal individual only an average of 2.35 m (95% CI 2.26-2.45, Archibald-Shaw, 2009), and spatial

autocorrelation (e.g., a positive correlation between the occurrence of alleles) extended out to a distance of 38 m. In this population if pollen movement is spatially restricted, then mating individuals are most likely related. Also, in this population the probability of full-sibship (r_p) among seeds is three times greater on mothers who are in canopy gaps when compared to families in closed canopy forests (Gardiakos, 2009). All of these results suggest that dispersal of both pollen and seeds in this system are spatially restricted.

Scaling up spatially the genetic differentiation of sampled pollen pools across alternate maternal individuals is quantified using two different indirect approaches. First, the Two-Generation Analysis of Pollen Structure (hereafter TwoGener) uses the set of pollen donor haplotypes as populations to quantify overall genetic differentiation (Smouse *et al.*, 2001) using a random effects multivariate linear model (Dyer *et al.*, 2004). This analysis assumes that maternal trees that are in close spatial proximity will sample from a more similar pollen pool and leading to a less differentiated set of pollen donors than those mothers who are further apart (Smouse & Sork, 2004; Smouse *et al.*, 2001). When the mother trees are sampled at close, moderate, and distant frequency from one another, the change in pollen donors contributing to different pollen pools (and resulting changes in differentiation) can provide an estimate of the average distance of effective pollen dispersal (Austerlitz & Smouse, 2001). In addition to overall differentiation, this approach also provides an estimate of the effective number of male contributors to a mother tree (N_{ep} , Smouse *et al.*, 2001, Sork & Smouse, 2006). This value N_{ep} estimates the number of pollen donors that would be required in an idealized population that could produce the same level of differentiation among pollen pools as observed in the real data (and its inverse should be similar to r_p from the mating system). This parameter is sensitive to

uneven contribution by paternal trees so N_{ep} is considered to be smaller than the actual number of potential males contributing to the pollen pool (Smouse & Sork, 2004).

A second approach is that of KinDist that focuses on pairwise relatedness of offspring produced by alternate maternal trees. Under limited pollen dispersal, there should be a higher probability of relatedness among offspring from spatially proximate maternal trees when compared to maternal trees separated by larger physical distances. Where TwoGener does not allow for certain assumptions such as flowering asynchrony or uneven male fecundity, KinDist can incorporate these parameters into the analysis, providing a more complete depiction of pollen pool genetic structure and average pollination distance. By incorporating both of these approaches, a better overall picture of the genetic structure of the population emerges (Robledo-Arnuncio *et al.*, 2006).

Despite the ubiquity of these indirect methods for quantifying contemporary pollen movement, they all assume that the intervening landscape is homogeneous and has no differential influence on the dispersal process. That being said, it is becoming increasingly apparent that landscape context both of where plants are located as well as the intervening landscape has a significant impact on genetic connectivity (Dyer *et al.*, 2010). In these analyses, two components are required: a representation of intervening landscape distances and a measure of pair-wise genetic distance. For the quantification of landscape distances, Least Cost Path (LCP) uses a cost (or resistance) surface to map movement, defined upon specific environmental variables. The shortest path between all sites (here that would be maternal trees) provides a measure of pairwise ecological distance across the landscape (Ray, 2005). Another method is based upon Circuit Theory (CT), where instead of the sole shortest path being used, the effects of all paths connecting sites are estimated (McRae & Shah, 2008). To provide analogous measures

of pair-wise distance for the genetic parameters, recent work in the Dyer Laboratory has shown that methods based upon conditional genetic covariance are significantly better at estimating the genetic parameters than pair-wise structure statistics (Dyer & Nason 2004, Dyer *et al.*, 2010). Given the rapid advancements in landscape genetic methodologies, the prudent approach would be one that compares the relative performance of alternative representations for both landscape and genetic estimators (e.g., Rayfield *et al.*, 2010, Dyer *et al.*, 2010).

The goal of this work is to determine the extent to which ecological context, both at the site of the tree as well as the intervening landscape, influences insect-mediated pollination in the understory tree, *Cornus florida* L. This work highlights the very first attempt at using landscape-sensitive approaches in the study of contemporary pollen movement. This project has two main objectives:

1. To use indirect analysis of pollen pool genetic structure to investigate contemporary patterns of gene flow in *C. florida* providing a baseline comparison to traditional currently published studies.
2. To use pollination networks based upon conditional genetic covariance and landscape sensitive approaches to understand how intervening landscape context influences landscape connectivity in populations of *C. florida*.

This study can help to bring the impact of the environment into population genetic analysis. For the first time researchers will be able to quantify how much selected environmental variables impact the genetics of a population. In doing this, the groundwork will be laid to help land managers and the environmentally conscious determine how to minimize human influence

on natural populations by identifying environmental variables that can influence gene flow in a population.

Materials and Methods

Study Area and Species: This study was performed at the Virginia Commonwealth University Walter and Inger Rice Center for the Environmental Life Sciences in Charles City County, VA, USA (Figure 1). The Rice Center is located on 138 hectares along the James River between Richmond and Williamsburg. The forest portion of the Rice Center is represented by *Quercus alba* (white oak) and *Pinus taeda* (loblolly pine) dominated hardwood forest with a mixed hardwood understory which includes the study species of interest *Cornus florida* L. (Cornaceae). Hurricane Isabel impacted this area in the Fall of 2003. The high winds and excess rainfall associated with the Hurricane blew down trees, changing the canopy dynamics and creating wind throws and gaps in the forest canopy.

The study species *C. florida*, also known as the flowering dogwood is one of America's most popular ornamental trees. According to McLemore (1890), the range of *C. florida* extends from extreme southern Ontario to east Texas, and south to northern Florida. In the early spring even before the leaves appear *C. florida* flowers (mid-March) in small, crowded, yellowish perfect blooms that are surrounded by four white to light pink petal-like bracts. This tree is insect-pollinated, and seed dispersal is usually by birds but can be propagated by gravity as well. The fruits of *C. florida* are clustered in bright red drupes that ripen from September to late October, and the fruit contains a two-celled, usually two-seeded, bony stone (McLemore, 1990). Individuals sampled in this project are part of a population that has been GIS mapped and used to quantify pollen mediated gene flow as part of the NSF DEB-0640803 grant awarded to Drs. Rodney Dyer and David Chan.

Sampling DNA Extraction and Amplification: Mother trees were selected across a range of canopy openings given that previous work showed significant differences in mating system parameters between open and closed primary canopies. Trees located in upland closed forest, lowland closed forest, upland wind throw, and lowland wind throw were selected and sampled haphazardly. Seeds were then collected from the mother trees. The seeds were removed from the fruit and the surfaces were cleaned. The seeds were then placed into individual air permeable bags using potting mix and cold stratified at 5°C for approximately 120 days (Young & Young, 1992). After germination, leaf material was collected from the offspring and stored at -20°C until DNA extraction at a later date. A total of 17 mother trees were sampled in an attempt to collect at least 20 offspring individuals per tree, producing 345 genotyped offspring.

The offspring and mother tree DNA was extracted using no more than 50 mg of leaf tissue using a DNeasy®96 Plant Kit (QIAGEN®Sciences Maryland 20874) and stored at -20°C. Four nuclear microsatellite loci developed from Cabe & Liles, (2002; Table 1) were used to genotype each individual mother and offspring. These loci were: Cf-G8, Cf-H18, Cf-N10, Cf-05. The primers were then tagged with the fluorescent labels HEX, TET, or FAM (IDT Bioscience) and used to amplify 10µL PCR reactions containing 2 µL 5× *GoTaq* Flexi buffer (Promega), 2 mM MgCl₂, 200 µM of each dNTP, 5% bovine serum albumin (New England Biolabs), 0.5 µM of each primer, 0.5 U *GoTaq* DNA Polymerase (Promega), and 1 µL template DNA. Genotyping was performed on a MegaBACE 1000 (Amersham Biosciences) following the manufacturer's recommendations, and genotypes were analyzed using the Fragment Profiler® software. Called genotypes were manually checked for both maternal individuals and their offspring.

Summary Statistics: For each locus, individual allele frequencies, the total number of alleles (A), the effective number of alleles (A_e), and the expected heterozygosity (H_e) were calculated using GeneticStudio (Version 3; Dyer 2009). The parameter A_e provides a measure of allelic diversity corrected by the frequency such that rare alleles do not disproportionately influence diversity. Expected heterozygosity is the frequency that is expected if the population were in Hardy-Weinberg equilibrium (e.g., large population size, random mating, no mutation, no migration, and no inbreeding) giving a measure of genotypic diversity (and is analogous to the Shannon-Weaver index commonly used in ecology).

Plant Mating System Parameters: Mating system parameters were estimated using the offspring genotypes and corresponding maternal genotypes using MLTR (version 3.2, Ritland, 2002) software. The multilocus selfing rate ($s_m = 1 - t_m$), biparental inbreeding ($s_b = t_m - t_s$), and correlation of paternity (r_p) were estimated using maximum likelihood under a mixed-mating model (Ritland, 2002). Statistical confidence for mating system analysis, paternity, and inbreeding statistics were determined by bootstrap analysis using 1000 replicates of individuals within families because family sizes and numbers were unequal. Another analysis was performed giving individual mother-level estimates of t_m and t_s , for which researchers can derive s_m and s_b for each individual mother tree.

Pollen Pool Structure: Both the TwoGener and the KinDist approaches were used to indirectly estimate pollen pool structure and contemporary gene flow parameters. The TwoGener analysis assumes that maternal trees that are in close spatial proximity will sample from a more similar pollen pool and have a less differentiated set of pollen donors than those mothers who are further apart (Smouse & Sork, 2004; Smouse *et al.*, 2001). When the mother trees are sampled at close, moderate, and distant frequency from one another the change in allele frequencies of offspring

can give an indication of the average distance of effective pollen dispersal. This analysis provides an index of differentiation (Φ_{ft}) estimating the amount of variation in the sample population that is due to differentiation among the sampled mother trees. The analysis was performed in GenAlEx (version 6.3, Peakall & Smouse, 2006). The total data and the pair-wise populations were permuted 1000 times. To determine differences in average relatedness sampled in alternate pollen pools (KinDist), the program POLDISP (version 1.0c, Robledo-Arnuncio *et al.*, 2007) was used to quantify the asymptotic reduction in pair-wise relatedness (Ψ) with increasing inter-mother distance. This analysis can also be used to estimate parameters such as effective male density (d_e) and Ψ both within and between each maternal sibship. In addition, KinDist also provides an estimate of the dispersal kernel wherein alternative distributional forms can be tested (Robledo-Arnuncio *et al.*, 2006). All dispersal distribution models (Normal, Exponential, Exponential-power, Geometric, and Bivariate Student's t-distribution) were used at varying threshold distances (300 m and 350 m). The smallest least squared residual error was used to select the model yielding the best fit for the data following the recommendations of Robledo-Arnuncio *et al.* (2006).

Site-Specific Ecological Variables: Several site-specific ecological variables were measured at the location of the maternal tree to investigate the possibility that local conditions are influencing mating parameters. During the pollination season semi-hemispherical photos were taken at the four compass points at the edge of each canopy for individual mother trees using a fish-eye lens on a Canon EOS Rebel digital camera (Chapter 14 Water Quality Monitoring Technical Guide Book, 1999). The percentage of sky pixels versus non-sky pixels and the degree of vegetative clumping above the mother trees was determined in Can-eye (Figure 2, Version 5; Jonckheere *et al.*, 2004, Weiss *et al.*, 2004). Percent sky pixels provides an estimate of the openness of the

canopy above individual mother trees which was shown in previous research to play a significant role in genetic variation of the population of *C. florida* at the Rice Center (Gardiakos, 2009). Clumping factor was also obtained in order to quantify the composition of the canopy above individual mother trees. For example, if the canopy above an individual mother tree has leaves and branches diffusely arranged compared to a mother tree with half of the canopy above it densely covered, they may have the same percent sky numbers but the degree of clumping will differ. Maternal individual parameters were also measured, which included both the diameter at breast height (DBH) and total floral output (TFO). Total floral output was measured by counting the total number of bracts on a tree. Two counts were taken simultaneously and averaged.

These variables were used to determine correlations between mating system parameters and site-specific ecological variables or individual maternal characteristics. Estimates of N_{ep} (number of effective pollen contributors contributing equally to that mother to give the genetic profiles seen) were correlated to the site-specific variable listed above using a Pearson's product moment correlation (R version 2.6.2). N_{ep} can be calculated two ways. The first, r_p^{-1} , assumes adult-level inbreeding and may produce inflated values. It can also be determined for individual mother trees to correlate to the site-specific variables that have been obtained for this study (Ritland, 2002). Another measure of N_{ep} obtained from TwoGener: $2\Phi_{FT}^{-1}$ (Smouse *et al.*, 2001), has been theorized to give estimations of N_{ep} that may be smaller than the actual estimate. This value however, is only a global value and cannot be obtained for individual mothers. It will serve to give researchers a range of N_{ep} for the study. Researchers can also obtain the realized N_{ep} for the study site from dividing the total number of pollen donors by the total area of the study site. Additional parameters included correlated paternity (r_p) estimated on each maternal tree, multilocus selfing rates (s_m) and consanguineous mating (s_b).

Genetic Distance for Landscape Analyses: This study used conditional genetic distance (cGD), available from GeneticStudio (Dyer & Nason, 2004; Garrick *et al.*, 2009; Dyer *et al.*, 2010), as a measure of among pollen pool genetic distance for all landscape analyses. The parameter cGD estimates genetic distances simultaneously for the entire data set, rather than in a pair-wise fashion and has been shown to provide significantly more powerful estimators of spatial structure (Dyer, 2007; Dyer *et al.*, 2010). To estimate cGD, the paternal contribution was subtracted from each offspring genotype. The multilocus pollen haplotype was used to construct a Population Graph (Dyer & Nason, 2004), and cGD was estimated as the shortest path distance through that pollen topology.

Ecological Distance for Landscape Analysis: Several raster layers were used as base templates for the estimation of ecological distance matrices. First, a 10 m² digital elevation model for the study area (Figure 3) was obtained from the USGS National elevation dataset for use in ESRI® ArcMapTM (version 9.3.1 ArcInfo license level). The spatial distribution of coniferous vegetation (Figure 4) was obtained from the SDE_Rice database (Center for Environmental Studies, Virginia Commonwealth University). A raster representing the spatial coverage of individual *C. florida* canopies (Figure 5) was created by taking the average canopy size for each individual and creating a buffer around all georeferenced adult *C. florida* trees at the Rice Center. Two open-canopy rasters were created, one representing the roads (Figure 6) and another the large field (Figure 7). The roads raster represents open understory since most of the canopy has overgrown the roads. The field raster represents true open canopy since it is clear-cut every couple of years. These rasters were hand-digitized from a digital orthophoto quadrangle obtained in the winter of 1994 with a resolution of 1 m. The roads shapefile was then buffered with a 4 m buffer to represent the true width of the road. The remaining canopy cover that was not represented by

coniferous vegetation, dogwood canopy, roads and the field was classified as “Mixed Hardwoods” and obtained by subtraction. Finally, a raster representing Euclidean distance was created by straight-line distance across the study site (Figure 8). In total, the convex hull of the study site measures 61 hectares.

Cost matrices representing Euclidian distance, Coniferous Vegetation, Field, Roads, Dogwood Canopy, Mixed Hardwood, and Elevation were individually estimated. For each of these factors, several levels of resistance were used because researchers do not know *a priori* the extent to which the costs associated with crossing individual ecological types influence the insects as they move across the landscape. As a result, a range of relative costs were examined including 20:1, 10:1, 7:1, 5:1, 2:1, 1:2, 1:5, 1:7, 1:10, and 1:20 were used in the creation of resistance rasters. For example a 20:1 cost raster indicates a cost of 20 to move inside the ecological variable and a cost of 1 to move outside of the variable. This was performed for all of the ecological variable layers except Euclidian distance and elevation, yielding 52 predicted resistance surfaces. On each of these 52 predictor topologies, two approaches were used to estimate pair-wise ecological distance between all pairs of maternal trees. First, least-cost path analysis (LCP) was used to find the single shortest path through the cost surface separating individual maternal trees. These distances were estimated using PATHMATRIX (version 1.1; Ray 2005) in ARCVIEW 3.x (ESRI, 2010). The second approach for quantifying landscape was one based on Circuit Theory (CT). Pair-wise distances based upon CT were estimated using CIRCUITSCAPE (version 3.5, McRae & Shah, 2009). The correlation between distance estimated under both movement models was examined using R (version 2.6.2) using standard statistical measures.

Analysis of Landscape and Genetic Features: Quantifying the extent to which both LCP and CT provide the same relationships among maternal locations, distance matrices for the same environmental variable at the same level of resistance cost were tested using a Pearson product moment correlation in R (version 2.6.2). If the two matrices are highly correlated then the two alternate movement models do not deviate and either could be used in subsequent analyses. However, if the two methods for estimating ecological distances are not highly correlated then the two analyses are not giving the same explanation to how pollen is moving across the landscape and subsequent analyses must be used to determine which approach provides a better fit to the observed data.

Direct comparison of the ability of alternate movement models to describe observed genetic differences was performed using Mantel correlations (Mantel, 1967). Both distance matrices from the same rasters for both LCP and CT were correlated with the genetic distance matrix (cGD from above). If both analyses provide the same predictive power then the correlations should be the same and a plot of the correlation statistics for all environmental layers across all levels of resistance of LCP vs. CT would resemble a $x=y$ line. However, if one analysis has better predictive power then the correlations for that analysis will be higher than the other and the plot will either be above or below the $x=y$ line. It is also possible that for individual ecological variables, insects may follow one movement model whereas with other ecological variables they may follow another (e.g., all use the roads to trapline but when it comes to coniferous vegetation, they use many different paths through the understory). In this case, one would use the metric that provides the best individual correlation for that ecological raster type across all levels of resistance.

Once the best set of predictive measures was identified, their relative correlation with the observed distribution of genetic variance was determined using the Mantel and Partial Mantel tests (Mantel, 1967; Smouse *et al.*, 1986; Legendre 1993). A Mantel test is a measure of matrix correlation (represented by a genetic and several potential environmental matrices in this paper) that uses permutation to estimate significance. For the individual Mantel tests, all environmental factors and their associated resistance values were tested for correlations with the cGD matrix. Then the highest correlated matrix for each environmental variable was used for subsequent partial Mantel tests (e.g., a measure of the correlation between the genetic and environmental variables given the relationship already between an existing environmental variable). Partial Mantel tests are helpful in determining if two environmental variables are contributing identically to the observed genetic differences.

Results

Summary Statistics: All loci assayed had a high level of diversity resulting in a powerful discrimination amongst individuals. General summary statistics for each locus include: Cf-G8 had a total of 21 alleles (A), a total of 6.418 effective alleles (A_e), and an expected heterozygosity of 0.844 (H_e). Locus Cf-H18 had a total of 19 alleles (A), a total of 8.656 effective alleles (A_e), and an expected heterozygosity of 0.884 (H_e). Locus Cf-N10 had a total of 13 alleles (A), a total of 8.462 effective alleles (A_e), and an expected heterozygosity of 0.882 (H_e). Finally, locus Cf-05 had a total of 12 alleles (A), a total of 6.791 effective alleles (A_e), and an expected heterozygosity of 0.853 (H_e ; Table 2).

Plant Mating System Parameters: The multilocus selfing rate (s_m) was 0.015 and biparental inbreeding (s_b) was 0.113. The average correlation of paternity (r_p) was 0.118 providing an initial estimate for $N_{ep} = 8.47$. These results are in line with previous research performed in the Dyer Laboratory (Table 3, For previous years results see: Gardiakos, 2009). Individual mother-level estimates of s_m ranged from -0.014 to 0.119 and s_b estimates ranged from -0.094 to 0.326 (Table 4). These individual mother-level estimates will be used for further downstream analysis to correlate with individual mother-level environmental variables (Percent Sky, Clumping, DBH, and TFO).

Pollen Pool Structure: TwoGener analysis of the differentiation among pollen pools sampled by each mother was $\Phi_{ft} = 0.161$ ($P = 0.001$) indicating that 16% of all the genetic differentiation sampled from the pollen pools is due to differences between the mother locations. Previous

year's data are consistent with these findings ($\Phi_{ft} = 0.208$, $P < 0.001$, Gardiakos, 2009) as well as found levels observed for alternate trees in the Ozark Mountains ($\Phi_{ft} = 0.128$, $P < 0.001$; Sork *et al.*, 2005). Using $\Phi_{ft} = 0.161$ an estimate of $N_{ep} = 3.1$. Giving a range of N_{ep} 3.1- 8.5 and a realized N_{ep} of 8.016, which falls within the estimated range.

The relatedness coefficient (Ψ) estimates ranged from -0.206 to 0.187. The relatedness estimates evaluated across the range of inter-mother distances and exhibited the expected decay (Figure 9). The Pearson's product-moment correlation between these two values was $r = -0.31$, $P < 0.001$, indicating that as distance between mothers increases the probability that two randomly selected offspring share the same father contributor decreases (Robledo-Arnuncio *et al.*, 2007). This Pearson correlation is a linear parameter, but if there is no significant decrease in the among-sibship correlated paternity with distance, then the pollen pool genetic structure is probably too weak to allow for proper estimation of the dispersal curve (Robledo-Arnuncio *et al.*, 2007). The least squared residuals for the three alternative model curves (normal, exponential, and exponential-power) were used to determine the best-fit density function for the correlated-paternity values in order to resolve the effective male density (d_e , normal=10.164, exponential= 10.096, exponential-power= 9.362). The smallest residual indicates the best-fit line to the relatedness curve; therefore, giving the best estimate of d_e . Despite the differences in underlying models, unexplained residual variation across all models was very similar. The effective male density (d_e), was estimated from the model fit to the exponential-power function because it had the smallest residual value and found to be only 0.33 effective males per hectare. Effective male density could not be determined for the Geometric or the Bivariate Student's t function based on the expected correlated paternity decay for each as estimates of the shape parameter approached infinity (Robledo-Arnuncio *et al.*, 2007; personal communication with Dr.

Robledo-Arnuncio).

Site Specific Ecological Variables: Percent sky measured above each of the maternal individuals ranged from 18-50% sky pixels versus non-sky pixels (n=15). The amount of vegetative clumping above individual mother tree canopies ranged from 0.45-0.65 (n=15). Diameter at breast height (DBH) ranged from 1.72-10.92 cm (n=15). The last site-specific variable obtained total floral output (TFO) ranged from 2-730 (n=15).

There were no significant correlations between the amount of open canopy above maternal trees and mating systems parameters N_{ep} (Pearson $r = 0.1818$, $P = 0.5166$), s_b (Pearson $r = 0.3454$, $P = 0.2074$), and s_m (Pearson $r = 0.3972$, $P = 0.1427$). In addition to the total amount of open canopy above individual trees, the clumping of canopy openings was also estimated. The estimated extent of clumping in canopy opening had no relation with N_{ep} (Pearson $r = 0.0913$, $P = 0.7463$) or s_b (Pearson $r = -0.2004$, $P = 0.4739$) but was significantly negatively associated with s_m (Pearson $r = -0.6671$, $P = 0.0066$; when adjusted for outliers $r = -0.4379305$, $P = 0.1173$; Figure 10 and 11). This reduction in correlation and significance is due to the small sample size $n = 15$ being sensitive to outliers. Individual tree sizes as estimated from both DBH and total floral output (TFO) were also independent of individual maternal reproductive parameters (N_{ep} , $r_{DBH} = 0.3548$, $P = 0.1945$, $r_{TFO} = 0.3335$, $P = 0.2245$; s_b , $r_{DBH} = -0.1424$, $P = 0.2245$, $r_{TFO} = 0.2072$, $P = 0.4587$, and s_m , $r_{DBH} = -0.1407$, $P = 0.617$, $r_{TFO} = 0.2158$, $P = 0.4399$; Table 5).

Genetic Distance for Landscape Analysis: Conditional genetic distance (cGD) values ranged from 17.8245 to 2.92485, approaching a normal distribution (mean= 9.477, sd=3.629; Figure 12). In the Population Graph representing the distribution of conditional genetic variance, the

maternal individual with the most connections (e.g., node centrality) was 646 with 5 and the individuals with the least connections were 647 and 701 with 2 (Table 6). The more connections an individual has, the more influence that individual has on the population.

Analysis of isolation by graph distance (Dyer & Nason, 2004; Dyer, 2007; Dyer, 2010) showed a significant positive correlation (Figure 13, Mantel $r = 0.477821$; $P < 0.001$). This is similar to the pair-wise relatedness (Ψ) because the relatedness as inter-mother distance increases relatedness decreases. Here, as conditional genetic distance (cGD) increases so does the physical distance. Both are describing the same phenomenon. This suggests that pollen movement is limited across the spatial scale sampled. Maternal individuals in close proximity have higher genetic covariance (and lower conditional distance as covariance and distance are inversely related, (Gower, 1966).

Ecological Distance for Landscape Analysis: Across all sets of environmental variables, the degree to which ecological distances calculated by both LCP and CT was correlated spanned a large range of values though all were significant (Figure 14). The lowest correlation between the set of individual ecological distances estimated from each of the movement models was for movement within *C. florida* canopies at a resistance of 20:1 (e.g., it was 20x more costly to move within *C. florida* canopies than outside; Mantel, $r = 0.4804$, $P = 0.001$). The largest correlation among estimated ecological distances was for the restricted movement through the field also at a rate of 20:1 (Mantel; $r = 0.9731$, $P = 0.001$). Plots of the correlations between both movement models are provided in the supplementary materials (Figure S1-S7).

In addition to quantifying the similarity of how alternate movement models estimate ecological distance, the Mantel test was also used to determine which model as well as which

level of ecological resistance has the closest relationship with observed genetic distances.

Estimated Mantel correlations for genetic distance and environmental variables were estimated using CT ranged from $r = 0.21 - 0.47$ with the majority between 0.40 and 0.45 (Figure 15). For movement models based upon LCP, Mantel correlations ranged from $r = 0.38 - 0.54$ with the majority between 0.45 and 0.55 (Figure 16). All correlations for both LCP and CT were significant ($P < 0.015 - 0.001$). In direct comparison when evaluating the relative performance of either LCP or CT movement models, models based upon LCP consistently produced higher Mantel correlations than those estimated from ecological distances under CT (Figure 17). These results suggest that for this system, and the heterogeneity in this landscape (see Rayfield *et al.*, 2010 for a discussion on this) LCP provides a better fit to the data and will be used in all subsequent analysis.

Analysis of Landscape and Genetic Features: Finally, the extent to which individual genetic differences among sampled pollen pools are correlated with individual components of the intervening landscape and the relative importance of these variables was determined using single and partial Mantel tests. The ecological variable whose distance between maternal trees had the highest correlation was roads at a cost of 1:5 (e.g., it was 5 times more costly to not travel on roads; Mantel $r = 0.542$, $P = 0.001$). The next most correlated individual variables was coniferous vegetation at a cost of 20:1 (it was 20 times more costly to travel inside of the coniferous vegetation; Mantel $r = 0.5146$, $P = 0.001$). These results suggest that open understory promotes insect movement. Once the ecological variable of highest correlation was fit, a partial Mantel (e.g., fitting the correlation after taking into account the correlation due to roads) showed that the next variable that produces the highest overall Mantel correlation was elevation (Partial Mantel $r = 0.4199$, $P = 0.001$). After elevation, mixed hardwood with a cost of 1:20 was the next

most important variable to fit along with the road raster (Partial Mantel $r = 0.3857$, $P = 0.001$).

Interestingly, models fit with rasters in addition to the single road raster produced lower overall Mantel correlations than that fit using only the road raster.

Discussion

By far, the most interesting result is that the highest correlated environmental matrix to the genetic matrix was the roads layer. Before this study the environmental variables insects were influenced by was unknown. This study indicates that the insects prefer to move along the roads rather than through the closed canopy forest. Insects that expose themselves to a complete open understory and canopy may experience an increase in the predation rate as opposed to the closed forest. However, with the canopy closed above the roads and the understory cleared, the insects may have a beneficial environment with cover from microclimate changes (light), and protection from predators, but with less restriction to movement, creating less energy expenditures than having to navigate through the closed canopy forest.

As shown in other studies, *C. florida* is predominately an out-crossing species. Here multilocus selfing was less than 2% ($s_m=0.015$), and a previous study in the same population found 4% selfing (Gardiakos, 2009, $s_m=0.040$). While the year-to-year variation is twofold at the Rice Center, it is consistent with the findings of Apsit *et al.* (2002; $s_m= 0.0000-0.019$) from the Ozark Mountains of southern Missouri. *C. florida* is a predominantly self-incompatible species (Reed, 2004) though the mechanisms through which this incompatibility is manifest have yet to be determined. Despite having low rates of selfing, the actual value can fluctuate year-to-year and throughout populations due to pollen limitation, consanguineous mating, and several other factors. That being said, this species is clearly a predominant out-crossing species, which may

not be surprising considering the evolution of the showy bracts and the effort put into blooming early in the season.

The observed rates of biparental inbreeding and correlated paternity ($s_b=0.113$; $r_p=0.118$) were also consistent with other studies of this species (Table 7). Normally high levels of biparental inbreeding can indicate restrictions to pollen movement (Dyer & Sork, 2001) if and when there is restricted seed dispersal, which is the case here with *C. florida* (2.35m). If pollen movement is restricted, as seems to be what these data are suggesting, pollen pools the mothers are sampling from will tend to have fewer father contributors comprising them. This will lead to more offspring with the same father contributor. Under restricted dispersal, the likelihood of related fathers contributing to the same maternal tree is also increased. This is because tree species such as *C. florida* exhibit spatial autocorrelation at shorter distances (individuals at closer distances will be more likely to be related). When pollen movement is restricted the pollen pools will tend to be segregated within the populations and only certain fathers will be contributing to a smaller number of mother trees. This will lead to an increase in correlated paternity compared to continuous populations where pollen movement is not restricted (Dyer & Sork, 2001).

When the difference in correlated paternity from randomly sampled offspring from pairs of mother individuals was plotted versus the difference in distance between those mother trees a significant negative correlation was observed. This is expected due to isolation by distance theory, which says that as distance between two individuals increases the likelihood of relatedness decreases (Wright, 1943). This can also indicate that the pollinators are restricted in their movement. If there were no restriction to movement, then the expectation would be that at any distance the same correlated paternity value would be possible. Because this is not the case,

and as distance increases correlated paternity decreases, then impediments to pollen movement must be present.

Genetic differentiation among pollen pools, as measured by the TwoGener model, also suggests restricted dispersal. A total of 16% of the total genetic variance ($\Phi_{it}=0.161$) can be a factor to pollen donors contributing to alternate maternal individuals. In addition, this is similar to other studies of this species (e.g., Sork *et al.*, 2005), the level of differentiation observed in these data also falls into what is expected from insect-pollinated species in general (e.g., *Albizia julibrissin*, Irwin *et al.*, 2003; *Centaurea corymbosa*, Hardy *et al.*, 2004; *Dinizia excels*, Dick *et al.*, 2003; *Symphonia globulifera*, Degan *et al.*, 2004; *Trillium cuneatum*, Gonzalez *et al.*, 2006). These results can be interpreted, as pollinators are not moving randomly through the population, random dispersal would homogenize the pollen pools and differentiation would not be present, rather, the set of individual pollination events that resulted in the observed pollen pools appears to be spatially restricted (Smouse *et al.*, 2001). It is important to point out that the TwoGener model assumes homogeneity of pollen dispersal and that the intervening environment has no differential effects.

As estimated under the KinDist model, the effective male density for the best model (the exponential-power function) suggests that the distribution of genetic variation observed in this study requires a small number of effective pollen donors (e.g., $N_{ep} = 0.33 \text{ hectare}^{-1}$). While the exponential-power function allows for more long-distance pollination events than the other models evaluated (Robledo-Arnuncio *et al.*, 2006), these results suggest that pollen movement throughout the study site is very diffuse, and there is little impediment on the insects pollinating *C. florida* (Hufford *et al.*, 2011). It is clear that these estimates underestimate true value for density and the inability to fit the other models causes concern about the validity of the KinDist

approach in this system for two reasons. First, the rest of the approaches that contemporary gene flow is being examined suggest the opposite pattern of gene flow through this population: one of restricted gene flow. An effective population size of $N_{ep} = 0.325$ individuals suggests a pollen donor every 3.07 ha would result in long-distance dispersal not being restricted. Second, the KinDist approach does not incorporate the interaction of any environmental factor and assumes that the environment plays no role in pollination and is a homogeneous factor over the entire study site.

When using a visual approach to indirect measures of gene flow, such as creating a network from cGD values, the population can be analyzed for measures of centrality to determine which individual mother trees have the most connections. This would give them an integral role in the population, because mothers with the most connections, such as 646 (5) in the population in this study, are contributing to more individuals in the population than others with fewer connections (647 and 701 with 2).

When plotting cGD values against distance a significant positive correlation is seen (Figure 13), as the genetic distance increases so does the physical distance between the two individuals. This is consistent with isolation by distance theory (Wright, 1943). Mothers who are closer in physical distance will tend to be closer in genetic distance as well. Due to factors influencing the movement of pollen in the understory. The pollinators will encounter less resistance from the environment the closer to the initial tree they are, but as they move away in distance, they will encounter more environmental resistances. The likelihood of pollinators to expend the least amount of energy while pollinating is very high, so the rationale that they would tend to move less distance, thus creating less genetic diversity within a smaller physical distance, holds true. This would also create greater genetic distance at farther physical distances because

the pollinators are not moving as far so the pollen pool contributors are changing over physical distance thus changing the genetic contributions from the fathers over that distance causing more genetic differences over physical distance. However, comparing cGD to physical distance does not indicate the environmental factors that are creating this dichotomy.

Previous work in this population showed a relationship between canopy opening above maternal trees and correlated paternity, where mothers in closed canopies had more unrelated offspring. Sampling for this study focused on a broad range of canopy opening as well as recording other potential co-variants that may influence reproductive output (canopy opening as percent sky, vegetative clumping, DBH, and TFO) Surprisingly, only clumping of the non-light portion of the canopy was related with s_m and the remaining mating systems parameters (N_{ep} and s_b) were independent of local conditions. However, upon further inspection, a single outlier (the clumping with maternal individual 598) accounts for the relationship and, when the clumping observation was removed, the relationship was no longer significant (compare Figure 10 and 11). Because *C. florida* is a long-lived perennial, it is unreasonable to argue that annual variation in mating system parameters may be driven by stochastic variation in the abundance or dispersal habits of pollinating insects. While temporal variation in mating system has not been the topic of much focus, it occurs in at least one more long-lived, insect-pollinated species, *Daphne laureola* (Thymelaeaceae) by Medrano *et al.*, (2005).

The main focus of this study was to quantify the extent to which landscape-genetic approaches can be incorporated into the analysis of contemporary pollen flow. By far, the greatest benefit that landscape-genetic approaches have to offer pollen analysis is the manner by which landscape context is quantified and included in the analyses. Until now, the analysis of pollen movement was conducted under the assumptions that both the dispersal process itself and

the environmental context through which dispersal occurs are both homogeneous and continuous functions. This is why under the KinDist model; the relationship is modeled under several well-known decay functions.

Presently, there are two approaches for quantifying ecological separation. For all environmental factors examined, the distances estimated among maternal trees using either LCP or CT both produced significant correlated responses to the genetic data (range 0.21-0.54). In order to determine if the LCP and CT resistance-to-movement analyses are giving the same explanation to the environmental variables, correlations between the two were performed. Although, many of the matrices exhibited co-linearity, a few matrices were exhibiting vastly less. The cf20_out1 matrix was the least correlated (Mantel $r = 0.4804$, $P = 0.001$) indicating that LCP and CT are giving different results for that matrix. The LCP matrix is only providing the path with the individual mother pairs least cost (Ray, 2005), whereas, CT indicates the average cost to move between pair-wise mothers (McRae & Shah, 2008). The elevation matrix also exhibited significantly less co-linearity ($r = 0.679$, $P = 0.001$). Both of these matrices have significant amounts of variation between the two different analyses most likely due to the immense amount of differentiation in the environmental overlay. For example, the *C. florida* vegetation layer has an vast amount of non-*C. florida* canopy space; this can cause CT to allow for more pathways of movement in that layer than LCP will. The same is true for the elevation layer. The elevation layer is a continuous raster layer, this means that CT can take many different paths through this layer and average them, whereas LCP will choose a single path with the least cost. A study by Rayfield *et al.* (2010), found that LCP routes can be sensitive to differences in relative-cost values among different landcover types and can also depend on the spatial structure of the landscape.

Independent of the method used to examine correlations between environmental factors and pollen pool genetic structure, LCP consistently outperformed CT in terms of providing higher correlations (Figure 17). As mentioned above, differences in the heterogeneity of the landscape can cause LCP and CT to produce different movement corridors through the different environmental landscapes. These differences will cause the correlations between LCP and CT to differ. So it is recommended that both LCP and CT should be examined simultaneously in future studies, as it seems that neither may be the “best” approach for all situations.

Across all environmental factors (and several resistance levels of each), Mantel correlations suggested that genetic co-variation was most correlated with the presence of roads through the understory ($r=0.54$). These results are consistent with the idea that insects prefer to travel along the open understory routes created by roads as they move through the forest. This is interesting because by moving within the roads, the insects are potentially exposing themselves to attack by predators and wind disturbance. However, the road is a wide-open space and movement through the air is not hindered by having to navigate through the forest understory. This relationship, however, could also be a confounding effect of the sampling scheme. The main spatial axis through the set of maternal trees sampled is aligned along the axis of the main road through the study site, which can be contributing to the road layer being the highest correlated. It is not clear at this point how to correct for this possibility given the current sampling scheme and will have to be addressed in future work on this system.

Partial Mantel tests were performed by first fitting the roads matrix to the genetic matrix, then fitting the additional environmental matrices giving the next highest correlation of the elevation layer. None of the partial Mantel models provided larger correlations than the model fitting the road layer alone. While, the change in elevation is not great throughout the study

region (0.53-13.82 m), there could be at least two reasons why elevation may still be important. First, to the insects moving through the understory this could be a significant disparity. Many of the trees sampled in this study are either on hills or at the bottom of gullies and insects may be following isoclines of elevation. Insects may prefer to stay at the same relative elevation when moving between the trees than to constantly expend energy on moving up and down through the understory. Second, the change in elevation could be driving differentiation in individual plant phenology (overall timing of flowering is driven by both temperature and dark cycle). In this population, the phenology of entire trees appears to be in synchrony, however, individual inflorescences within trees may be highly asynchronous (Dyer, in prep., Gardiakos 2009, Carr, 2010). Consequently, individuals may be flowering either earlier or later (higher open areas versus those found in the lower cooler sites); similarity in elevation may increase genetic covariance. What remains is to be able to mark and follow individual flowers rather than entire inflorescences to measure the degree of genetic covariance among individual pollen pools.

By testing the interplay between the environmental and genetic factors at work with *C. florida* at the Rice Center, this research can serve as a model analytical approach to developing a conservation plan for other species. This study can help biologists, forest managers, and land developers to identify plans to lessen the impact of humans on natural populations of plants and animals. Identifying natural impediments and areas to bolster gene flow is an important step towards lessening the impact of humans on natural environments. Managers can then plan development to maximize population stability of the target species and lessen the strain of human influences on natural populations.

This study can also serve as a model to study insect-mediated gene flow in other species of plants. Since this is the first to identify environmental influences of pollinating insects, many

more studies like this are needed to elucidate what environmental cues pollinators' use. This will develop a better understanding of the factors that affect pollination. These studies can then be used to identify factors affecting pollination with species that are endangered or populations that are highly fragmented. Helping scientists to implement conservation plans to better facilitate the maintenance of the population or species.

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Appendix A

Tables

Table 1.

Locus	Primer Sequence	Motif	Annealing Temperature	Fragment Size Range (b.p.)	H _E (H _O)
Cf-G8	F:*GCTGGTTGAATTATTTGAGG R: GGGATTAAAAGAAAGATGACG	(CA) ₂ (CT) ₁₆	53°C	152-190	0.90 (0.94)
Cf-H18	F:*CACGGATCCATACATCATCG R: CTCTCTTCCTCCACCCCAAC	(TG) ₂₀	62°C	84-123	0.92 (0.72)
Cf-N10	F:*TGATTGAATAACCTTTTGATGC R: GGATGCTTCAAATGTCAACG	(GT) ₁₇	57°C	178-206	0.79 (0.72)
Cf-05	F:*TATAGAAGGGCGCAATGAAC R: TCTAAGGGATGCCAAGTGTC	(TG) ₁₇	55°C	177-197	0.82 (0.82)

* Fluorescently labeled primers

Table 2.

	G8	H18	N10	05
A	21	19	13	12
A _e	6.417999	8.6564183	8.4626339	6.7912425
H _e	0.844188	0.8844788	0.8818335	0.8527515

Table 3.

Parameter	Estimate
Selfing (s_m)	0.015
Biparental Inbreeding (s_b)	0.113
Correlated Paternity (r_p)	0.118

Table 4.

Mother	s_m	s_b
478	-0.001	0.107
513	0	0.185
519	0	0.125
559	-0.004	0.01
572	0.119	0.326
598	0.119	0.105
600	0.016	0.13
643	-0.001	-0.024
645	-0.001	0.083
646	-0.002	0.136
647	0.019	0.23
648	-0.012	0.102
701	-0.002	-0.039
706	-0.002	0.291
711	0.046	0.056
712	-0.002	-0.003
719	0.011	0.146
805	-0.014	-0.094

Table 5

	<i>Nep</i>	<i>p-value</i>	<i>sb</i>	<i>p-value</i>	<i>sm</i>	<i>p-value</i>
<i>Percent Sky</i>	0.1818183	0.5166	0.3453644	0.2074	0.3971584	0.1427
<i>Clumping</i>	0.09127604	0.7463	-0.200396	0.4739	-0.6670875	0.006595
<i>DBH</i>	0.354758	0.1945	-0.1424448	0.6126	-0.1406841	0.617
<i>Total Floral Output</i>	0.3334609	0.2245	0.2072143	0.4587	0.2157652	0.4399

Table 6.

Node	Total
478	4
513	5
519	3
559	3
572	4
598	3
600	4
643	3
645	4
646	5
647	2
648	3
701	2
711	4
712	3
719	4
805	4

Table 7.

Species	r_p	Reference
<i>Rutidosia leptorrhynchoidea</i>	0.36-0.61	Wells & Young, 2002
<i>Pinus sylvestris</i>	Large Population 0.0-0.5 Small Population 19.6	Robledo-Arnuncio <i>et al.</i> , 2004
<i>Pinus piaster</i>	0.018	de-Lucas <i>et al.</i> , 2008
<i>Mimulus guttatus</i>	0.21-0.44	Ritland, 1989
<i>Centaurea solstitialis</i>	0.05-0.64	Sun & Ritland, 1998
<i>Rhododendron aureum</i>	0.24-0.41	Hirao <i>et al.</i> , 2006
<i>Araucaria angustifolia</i>	0.07-0.09	Bittencourt & Sebbenn, 2007
<i>Pachira quinata</i>	0.47-0.89	Fuchs <i>et al.</i> , 2003

Appendix B

Figures



Figure 1.

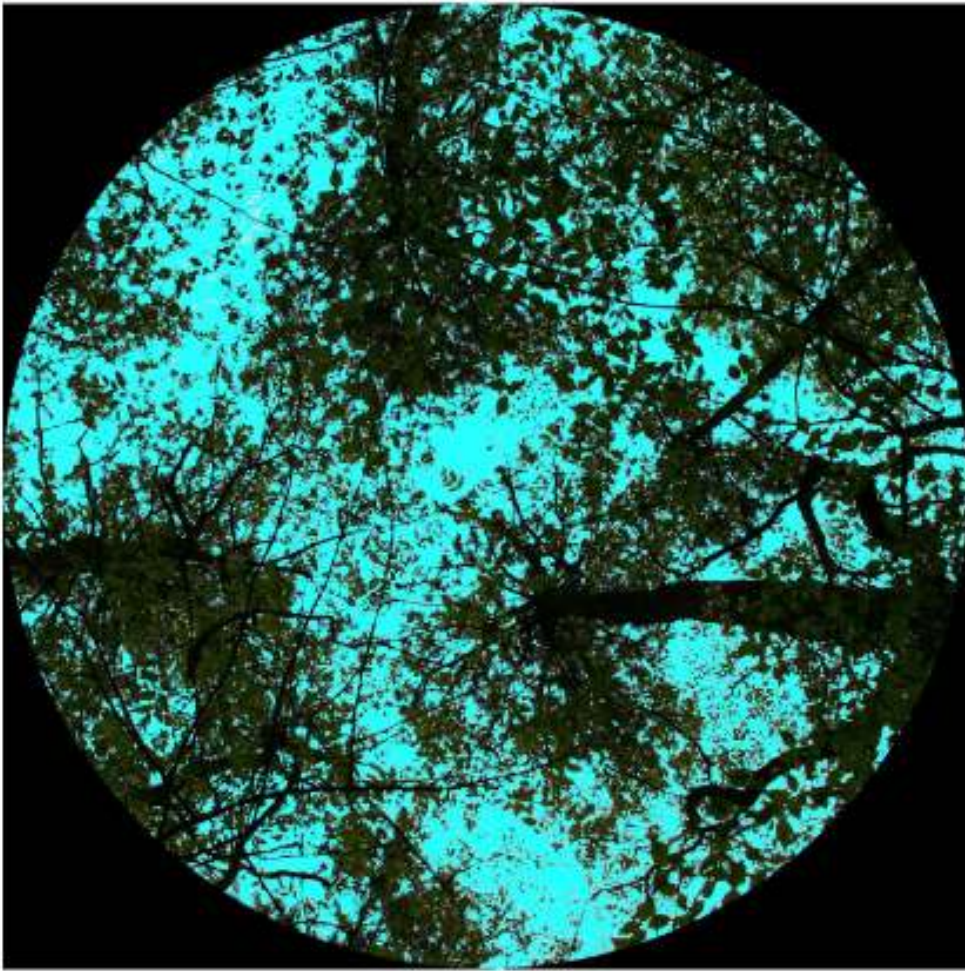


Figure 2.

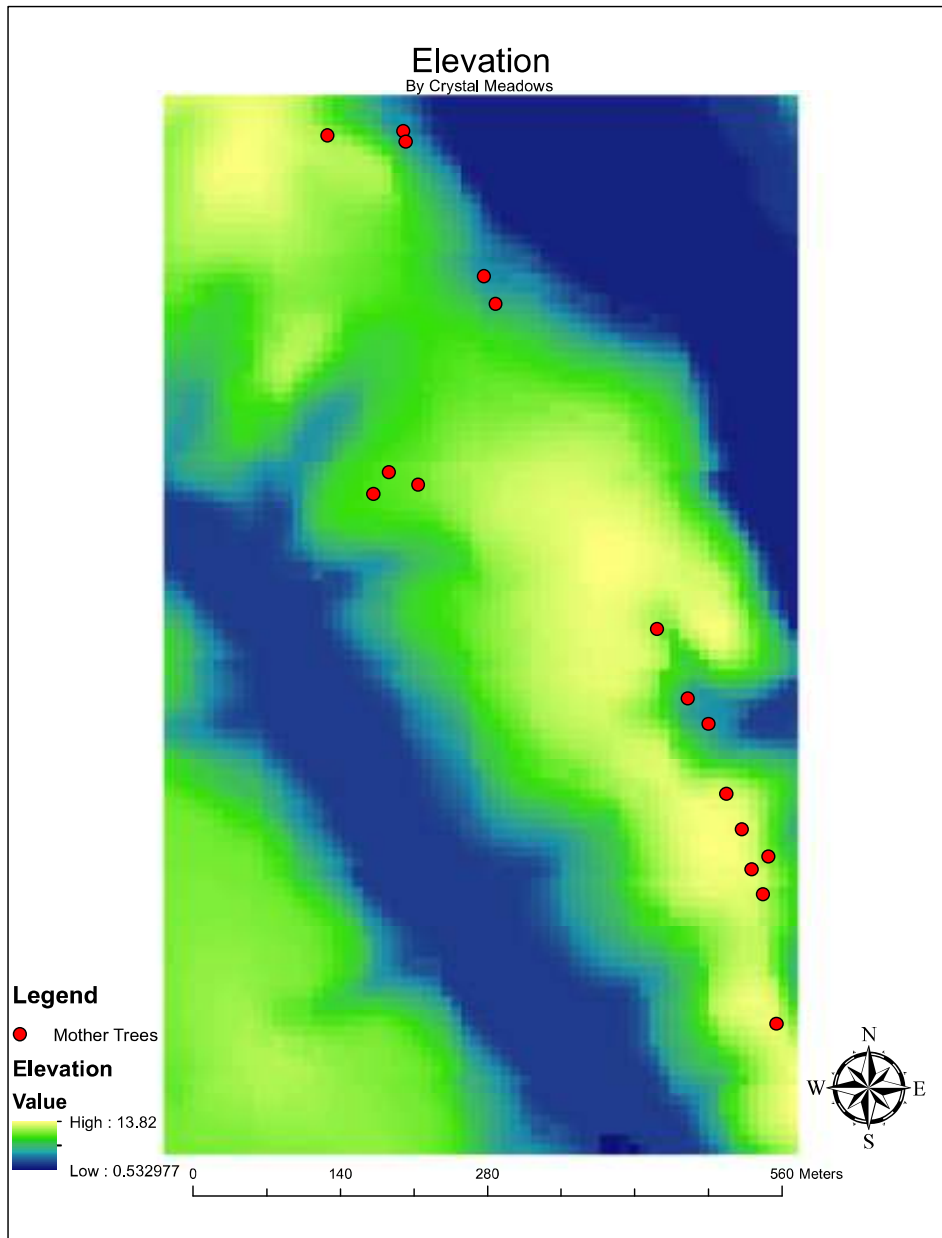


Figure 3.

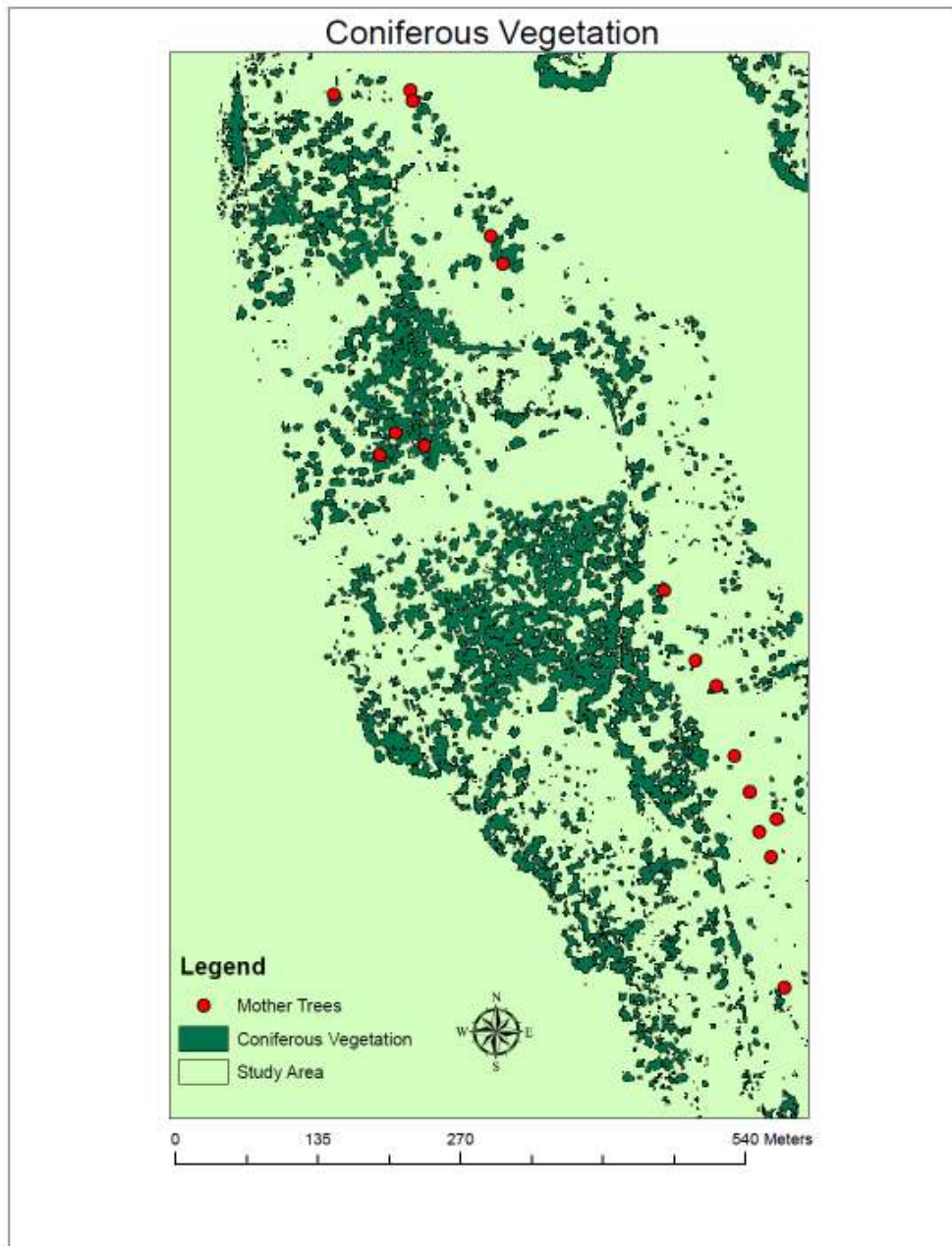


Figure 4.

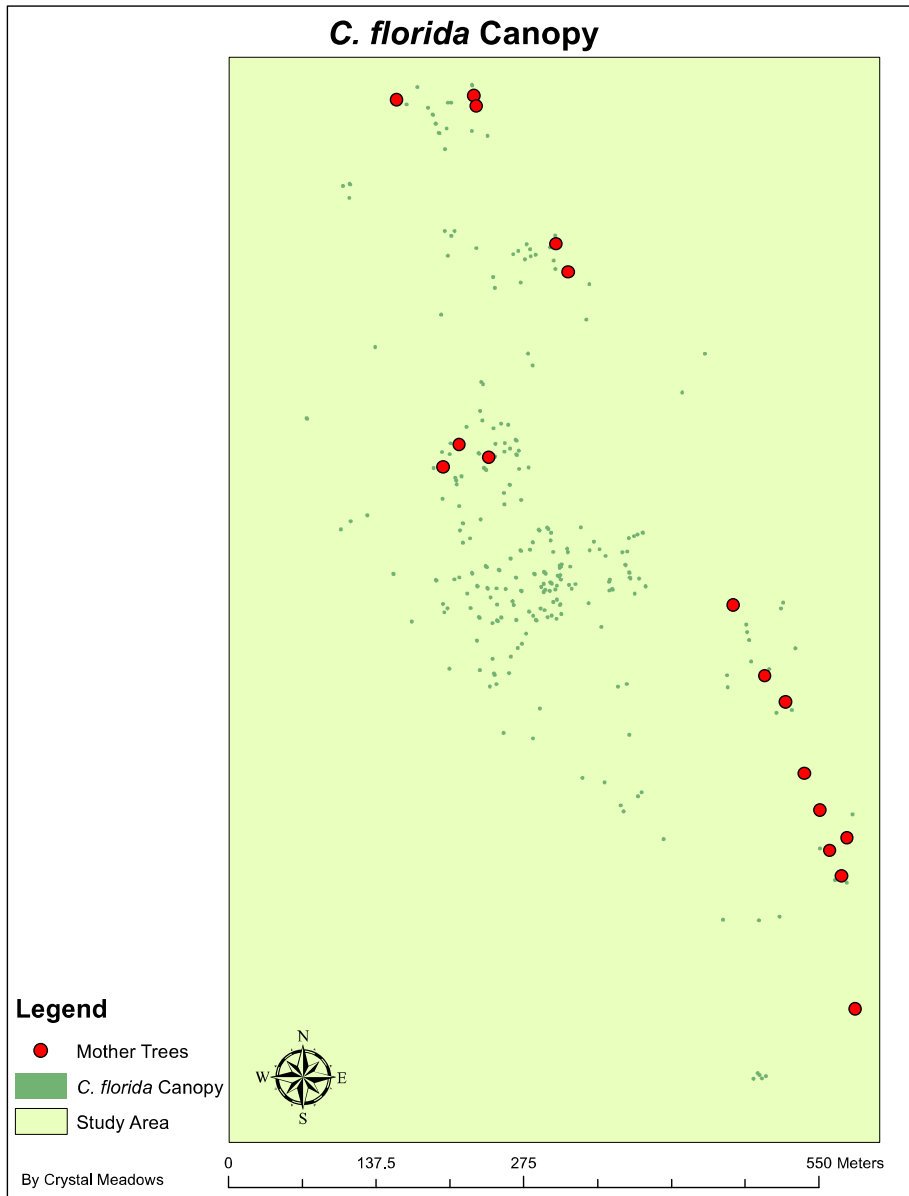


Figure 5.

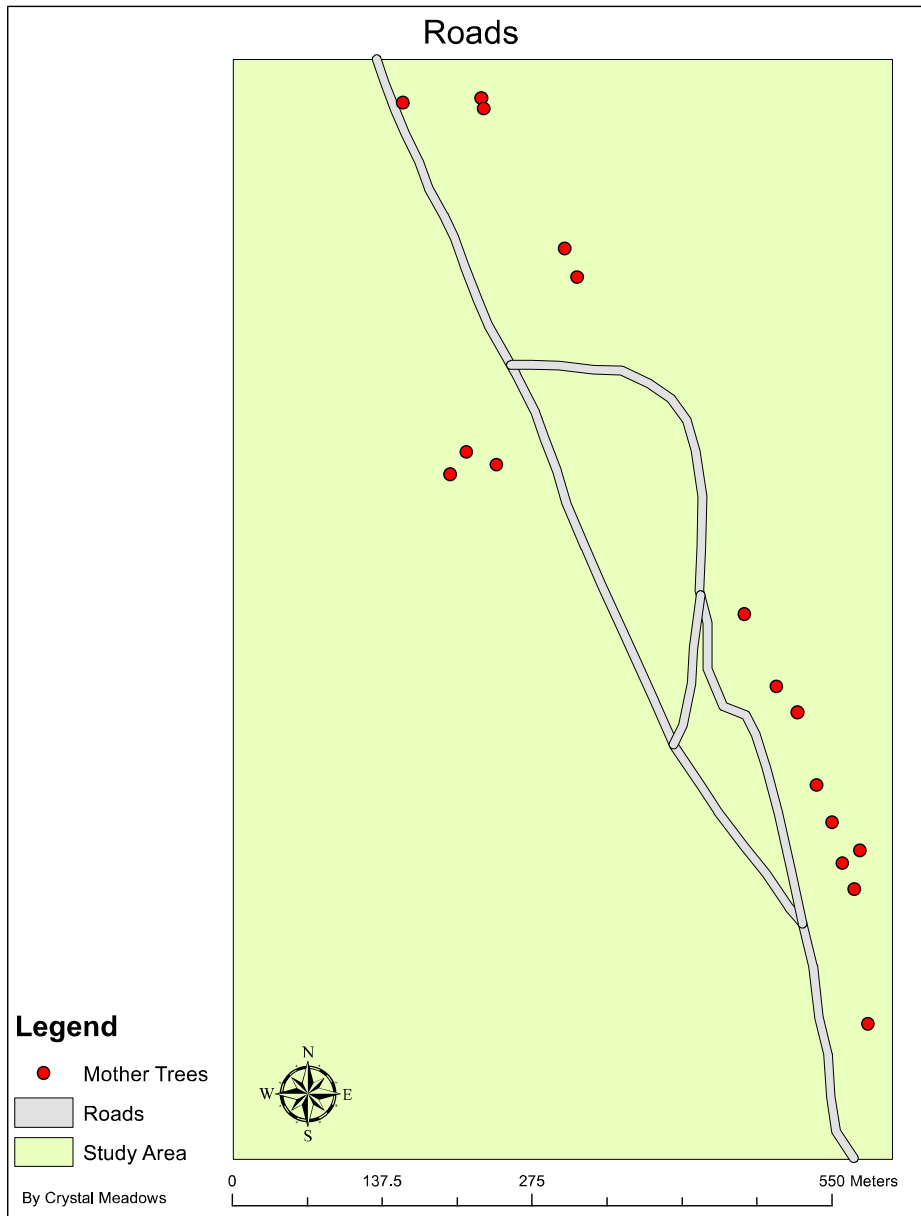


Figure 6.

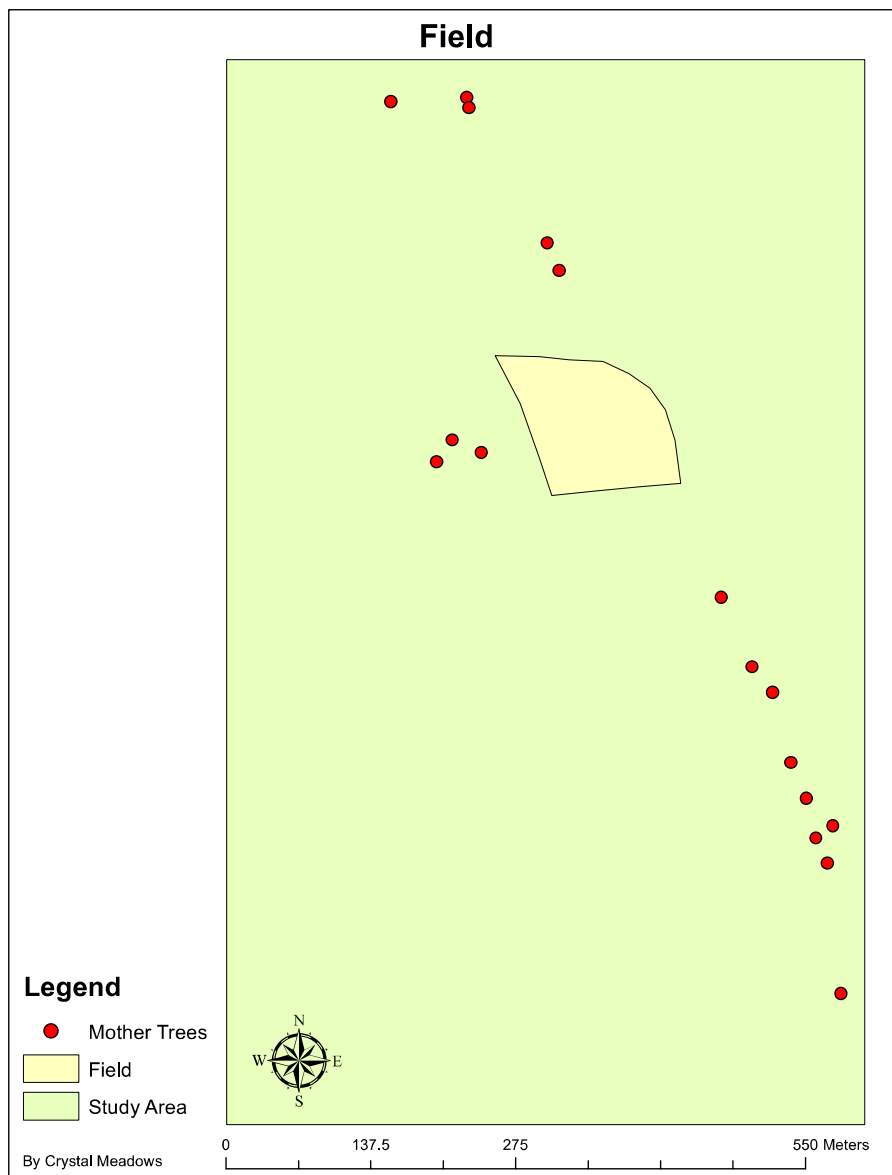


Figure 7.

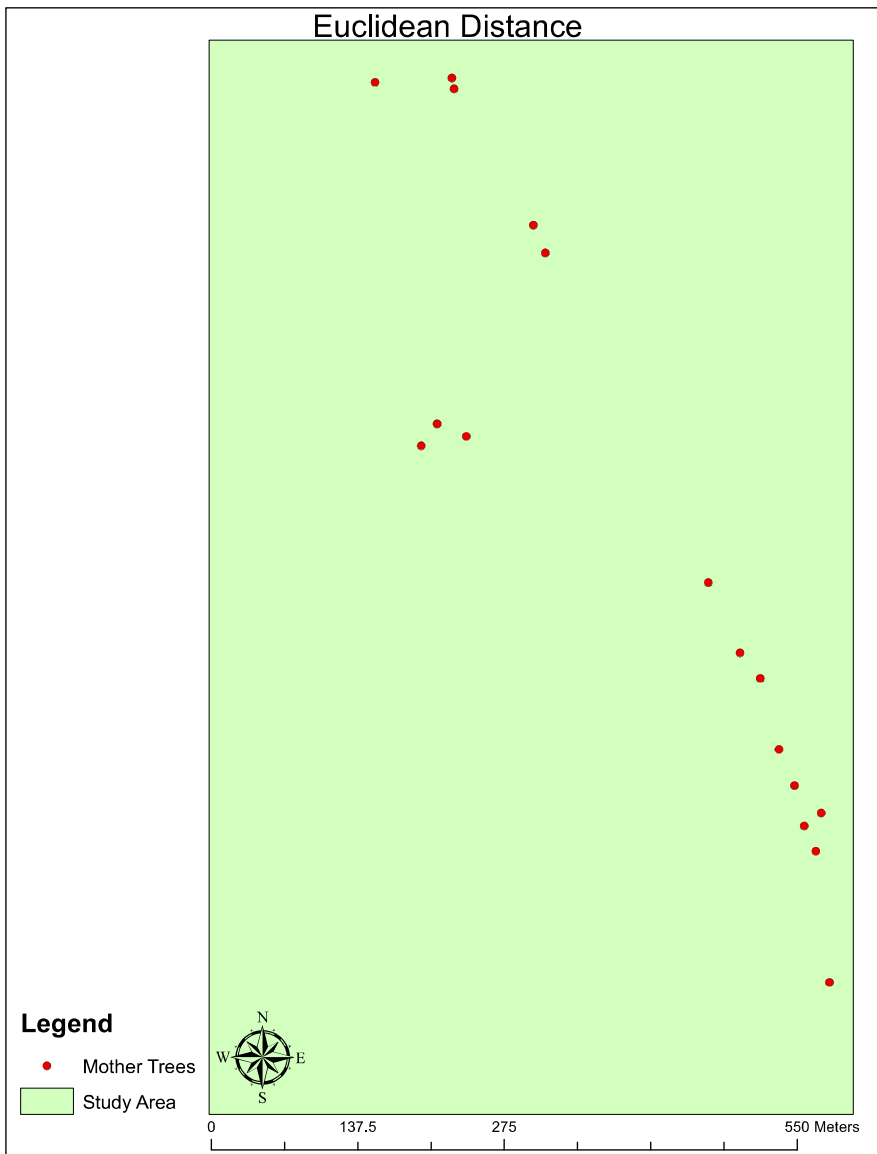


Figure 8.

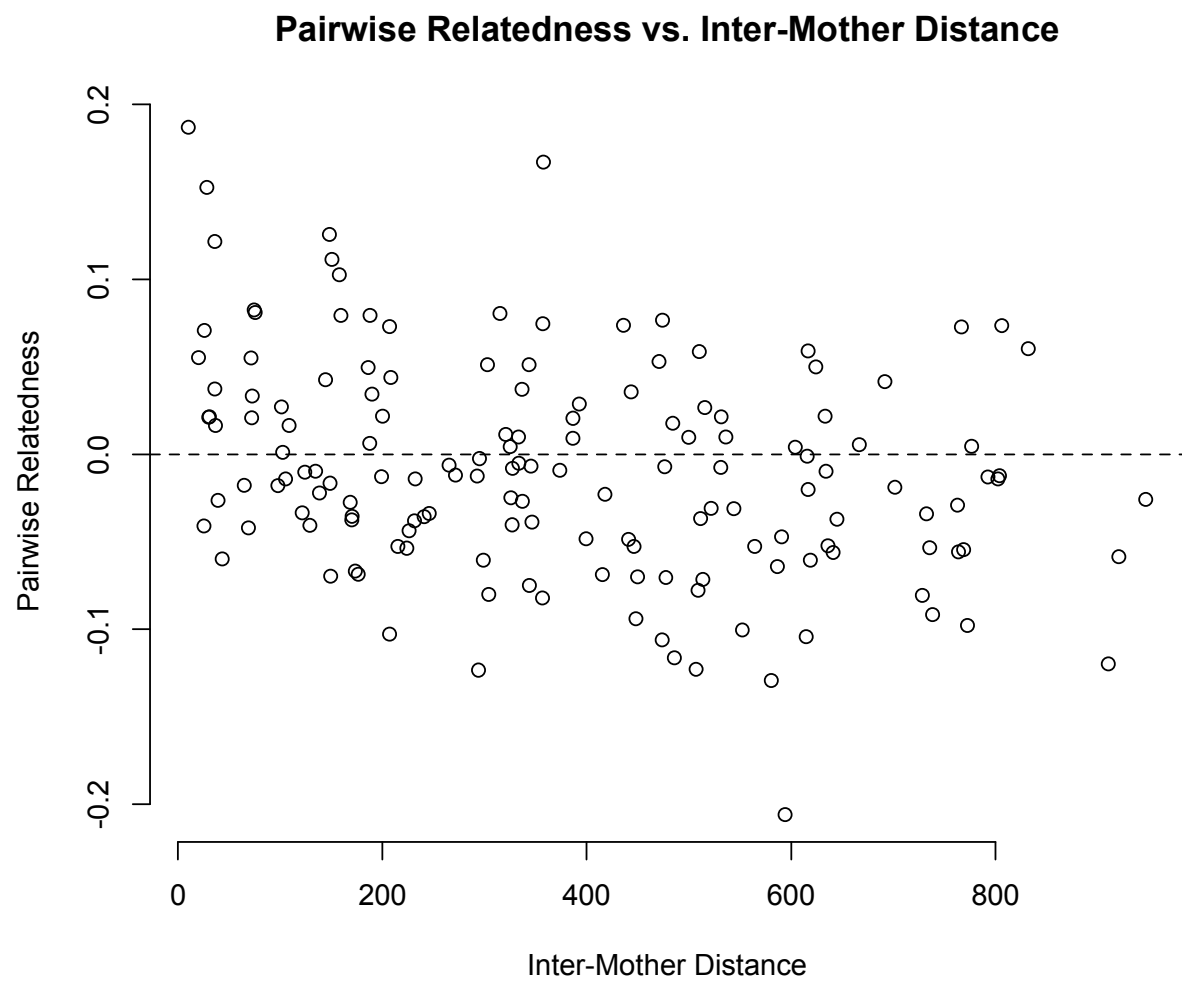


Figure 9.

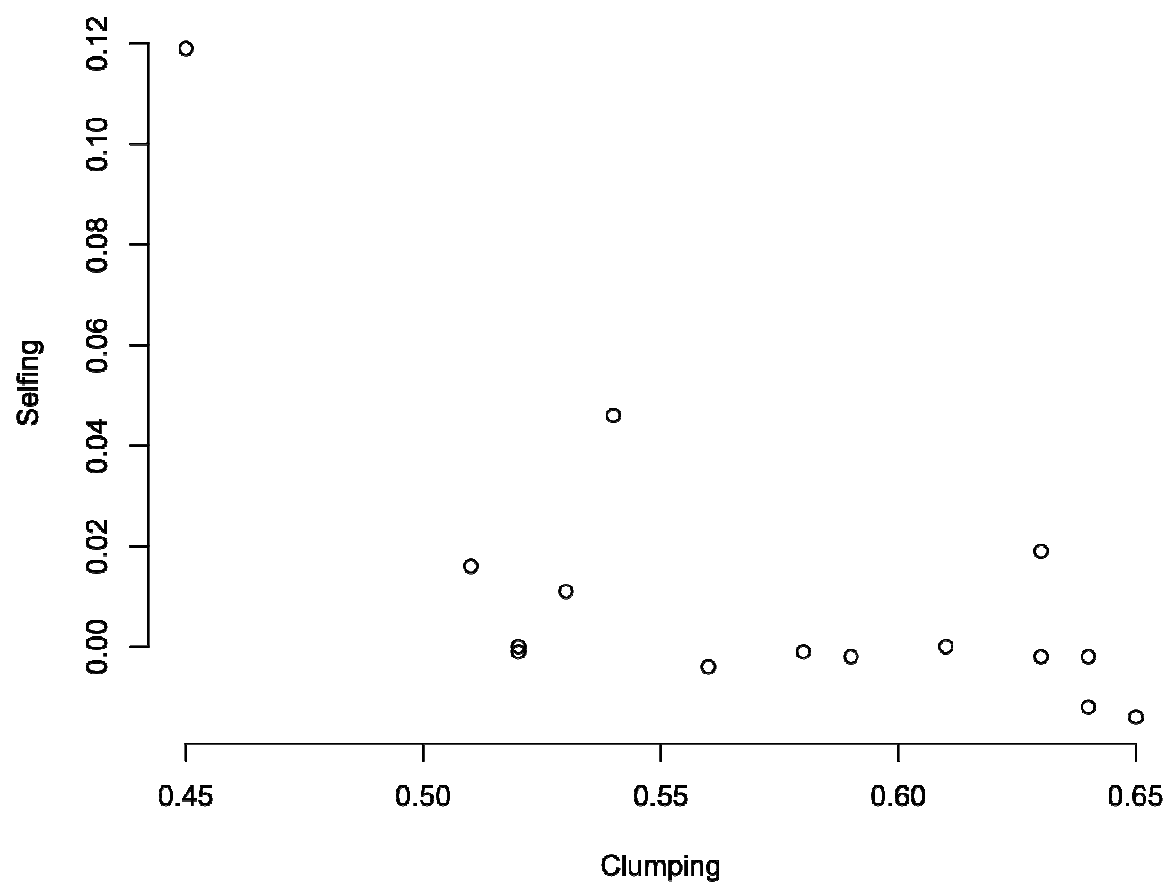


Figure 10.

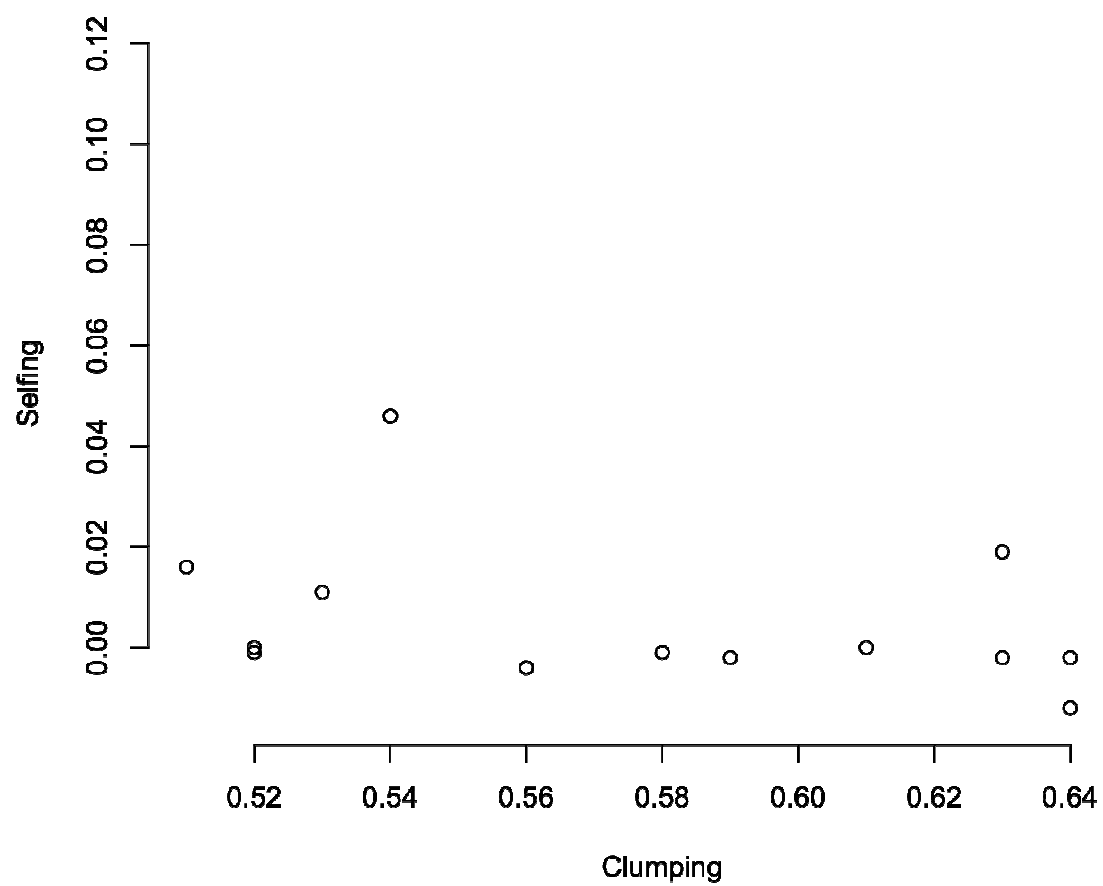


Figure 11.

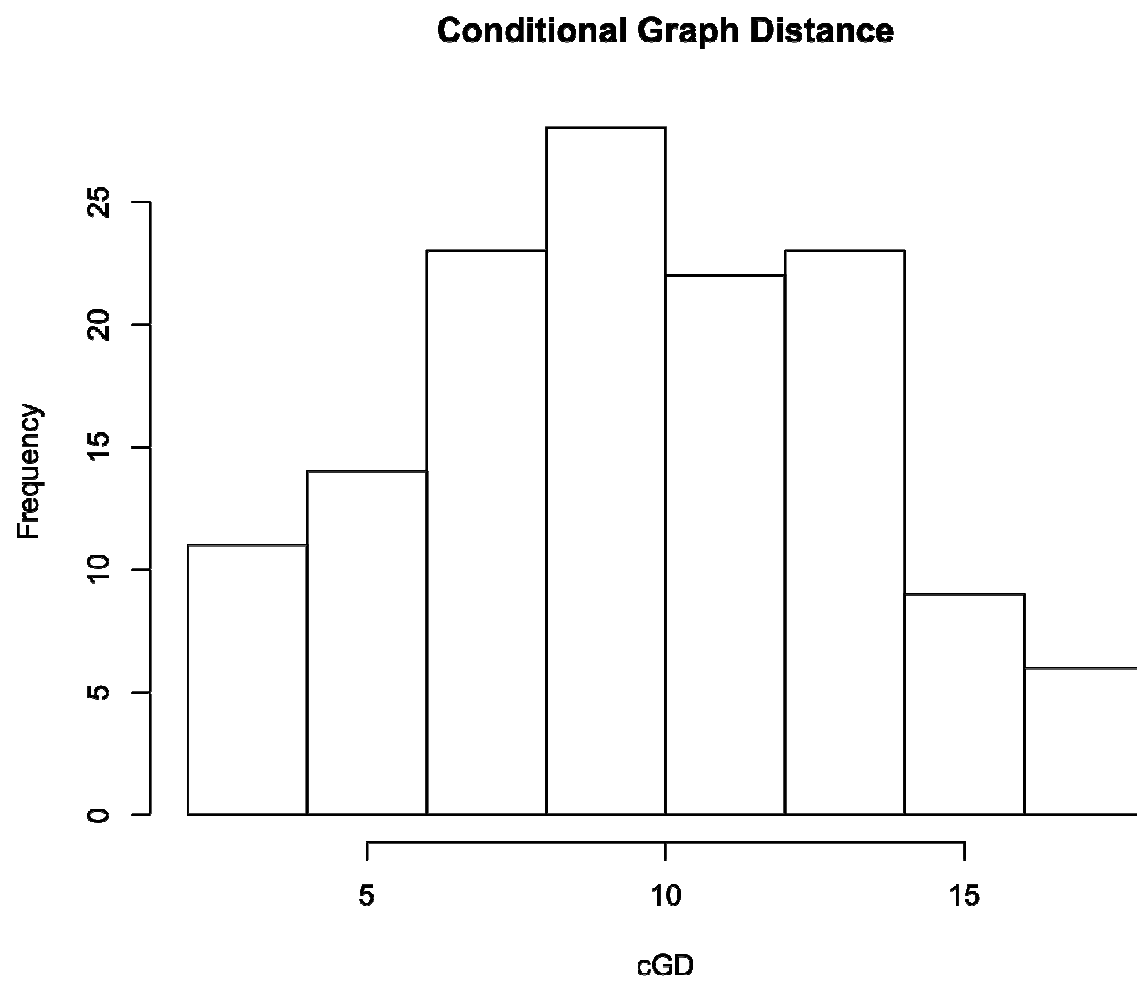


Figure 12.

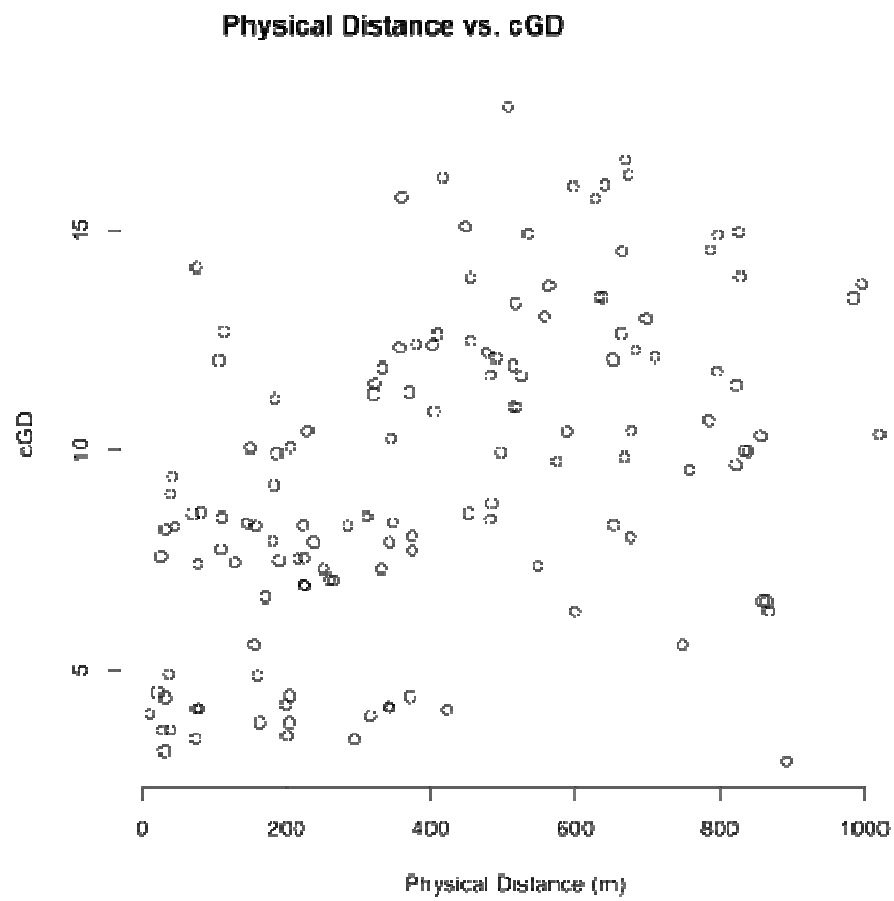


Figure 13.

Mantel Correlations for Environmental Matrices

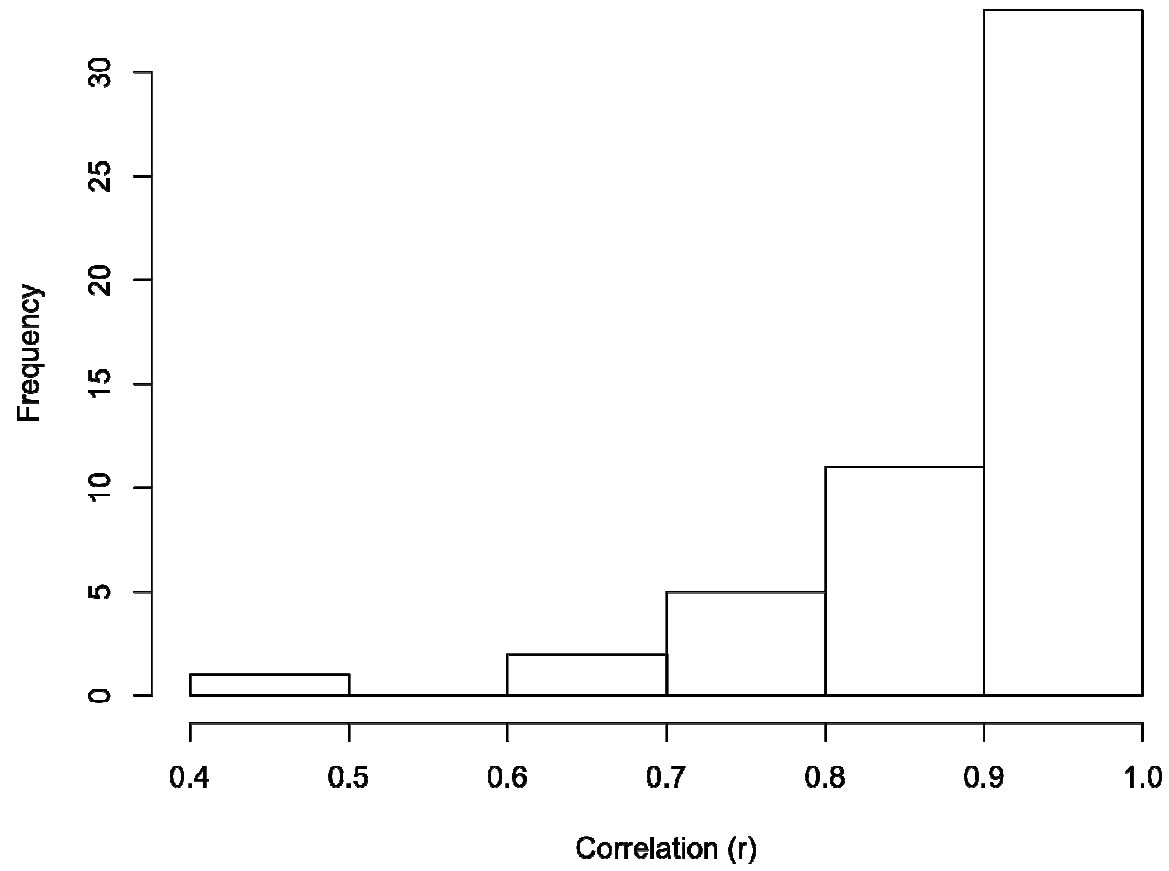


Figure 14.

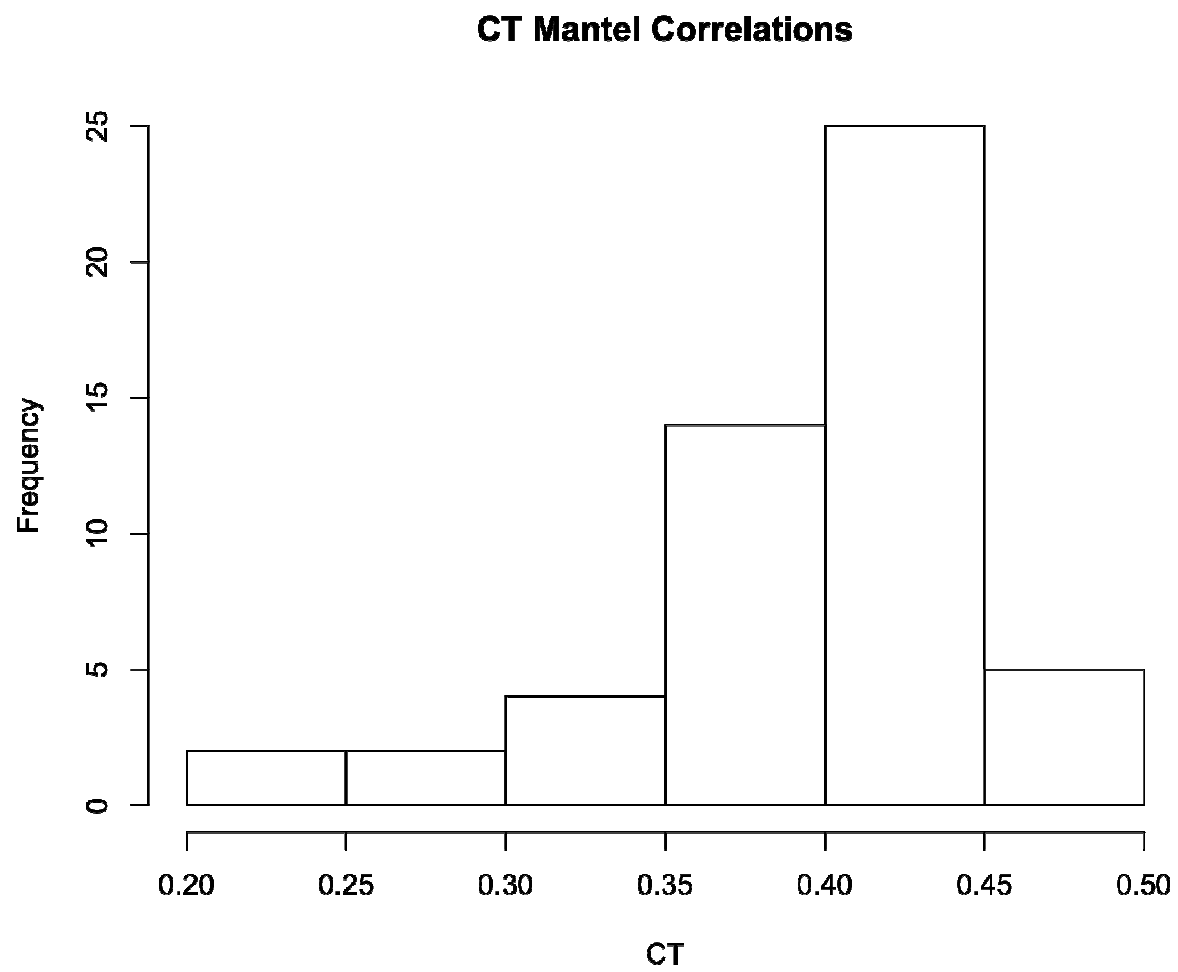


Figure 15.

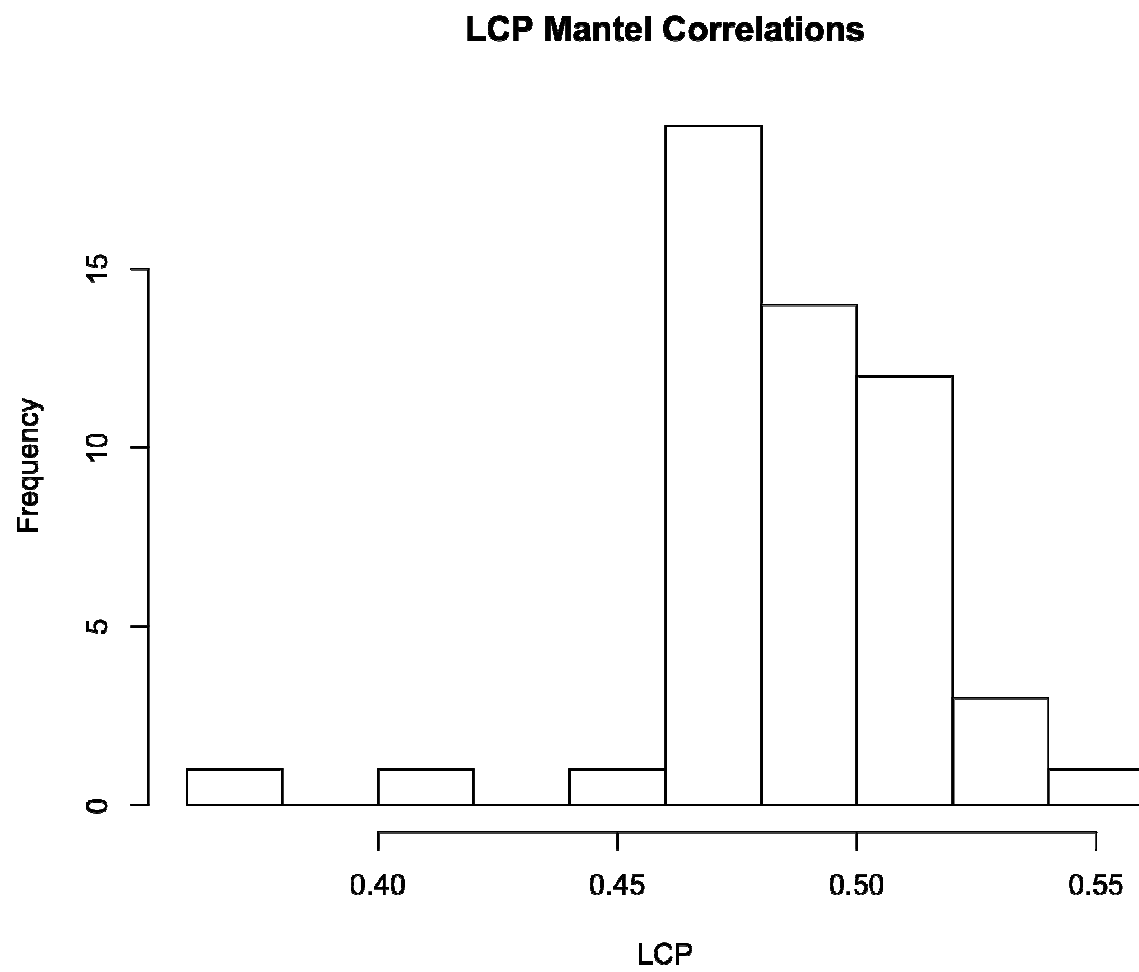


Figure 16.

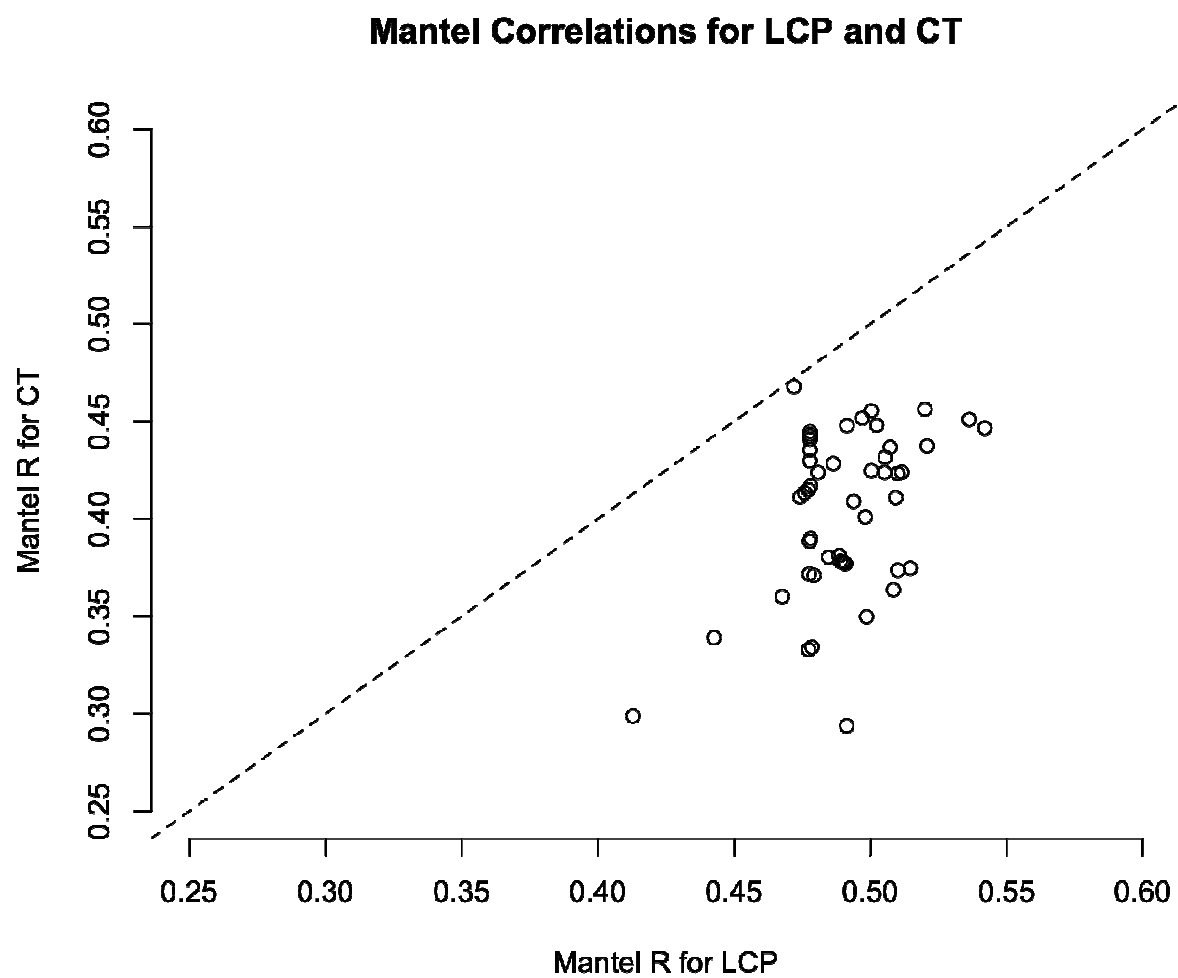


Figure 17.

Appendix C
Supplemental Figures

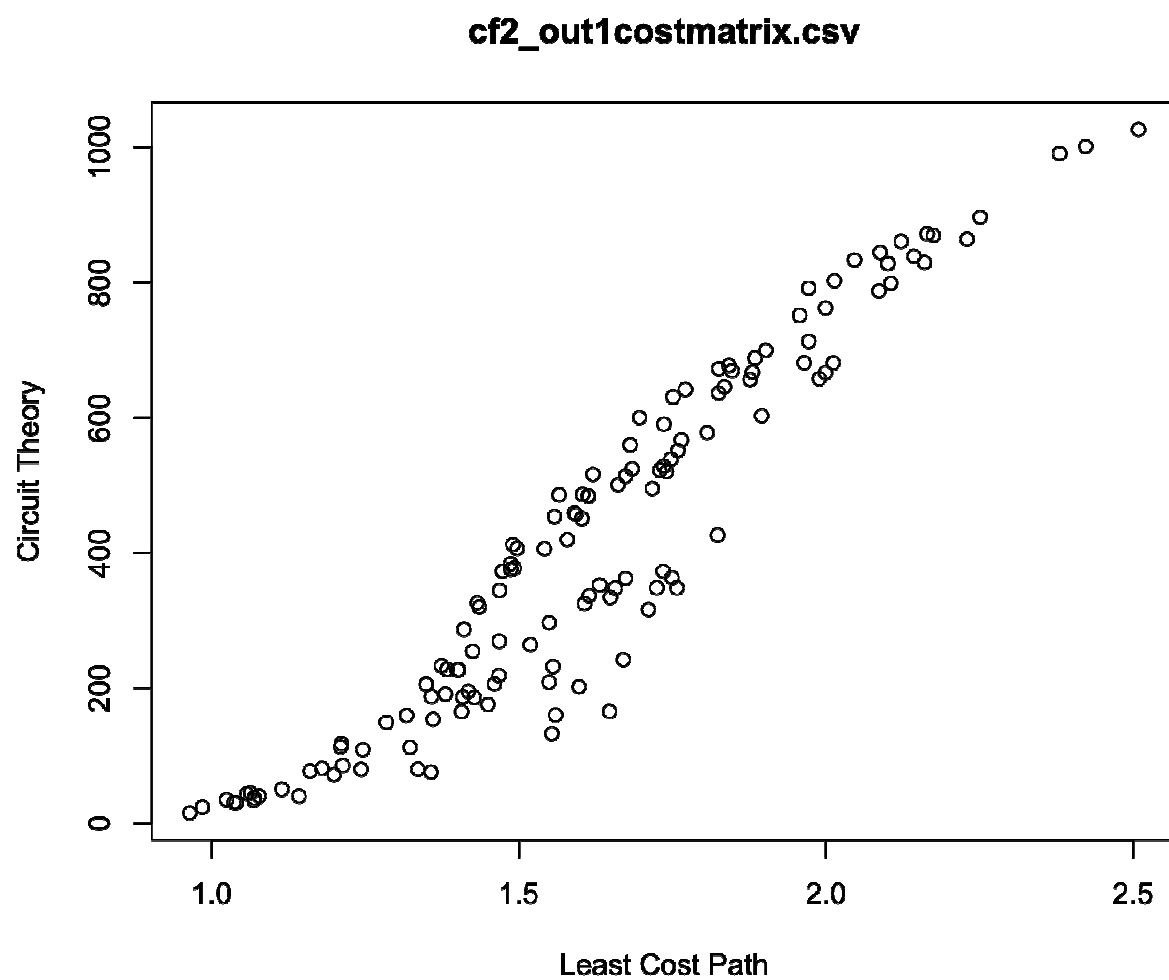


Figure S1.

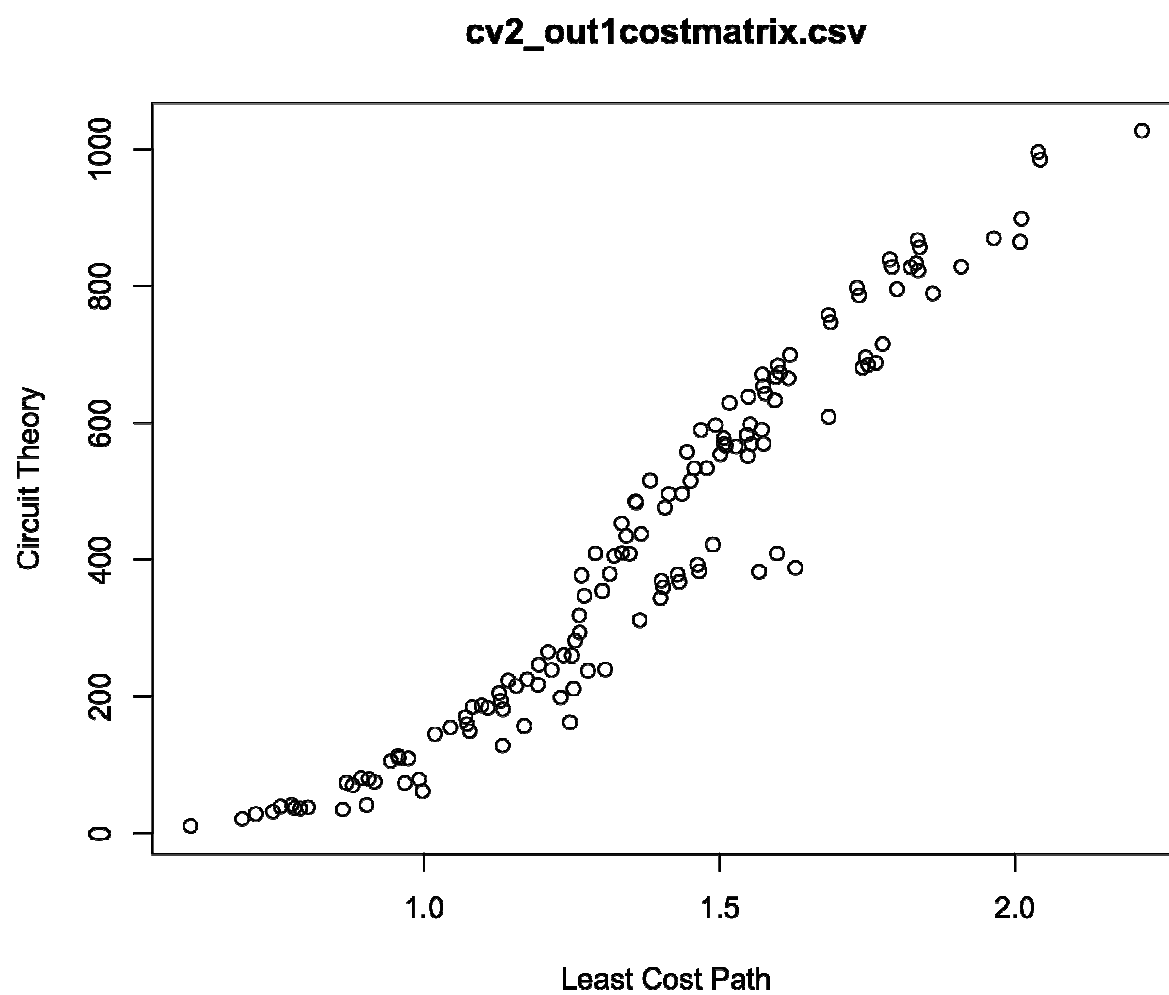


Figure S2.

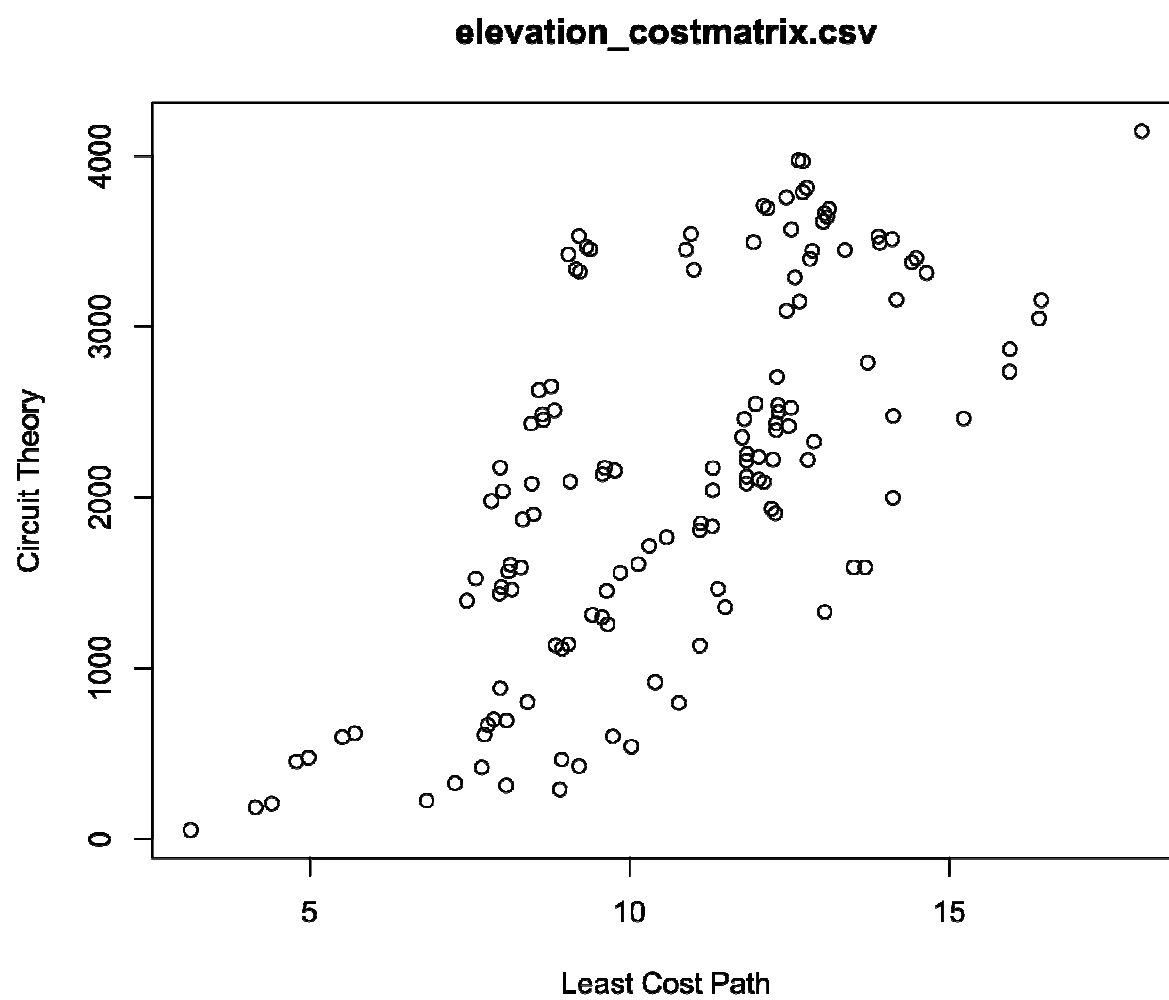


Figure S3.

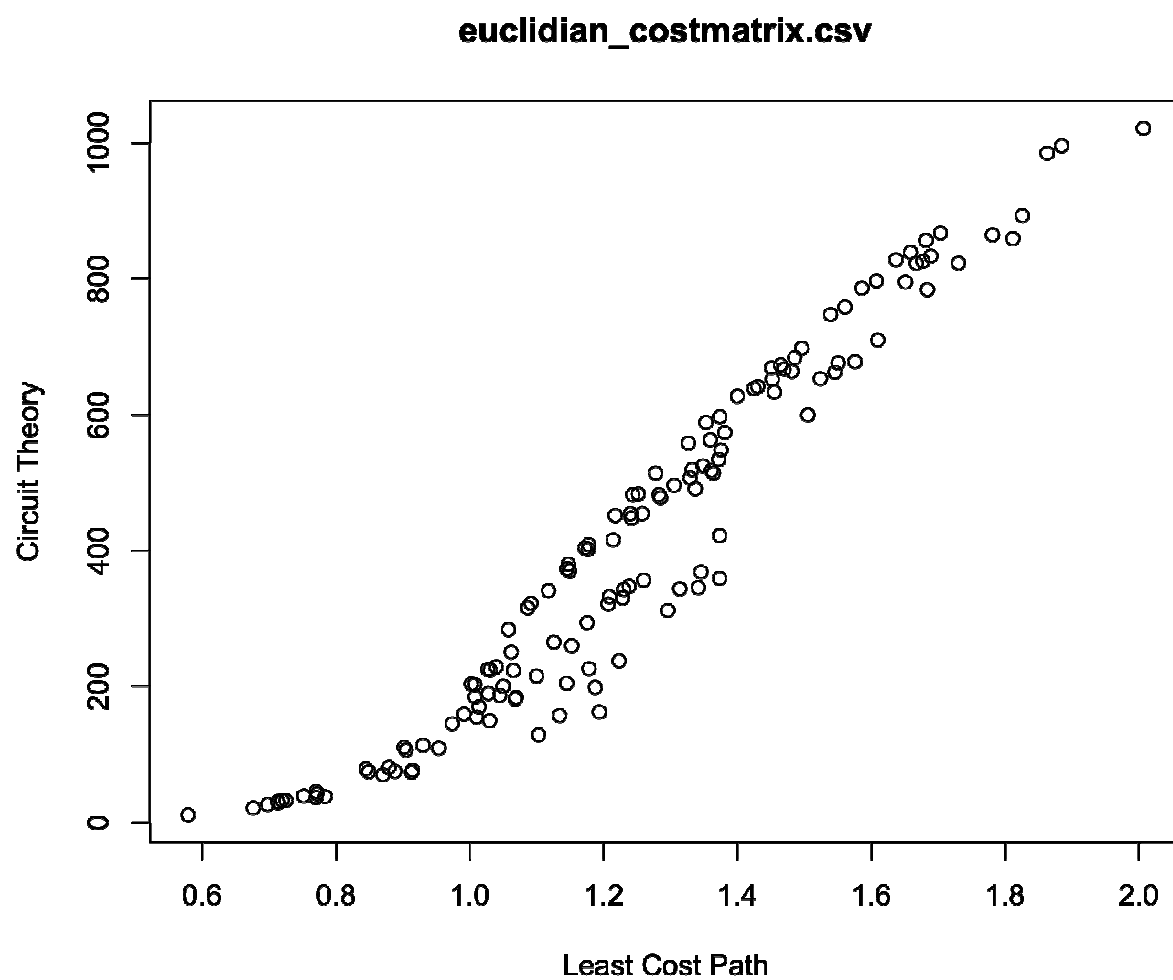


Figure S4.

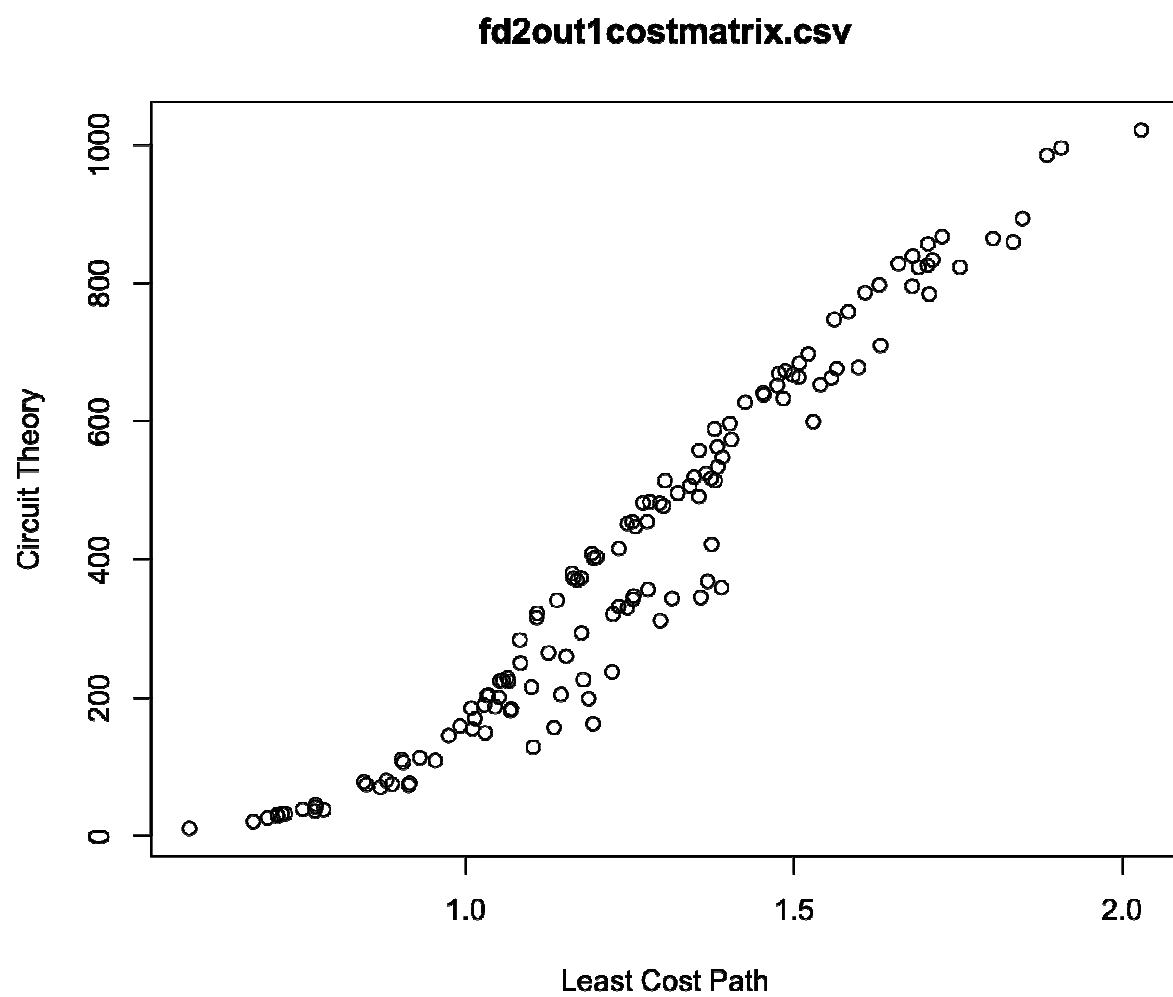


Figure S5.

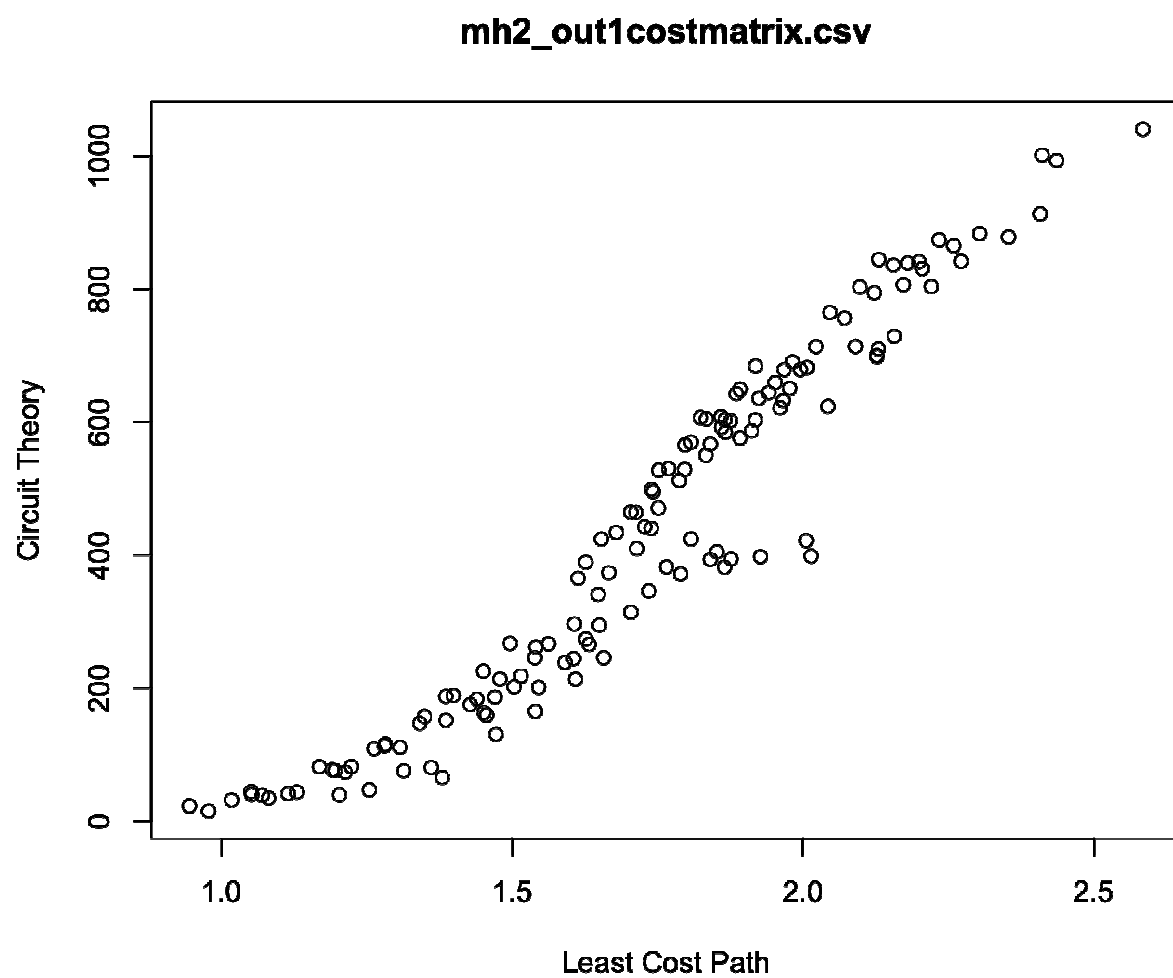


Figure S6.

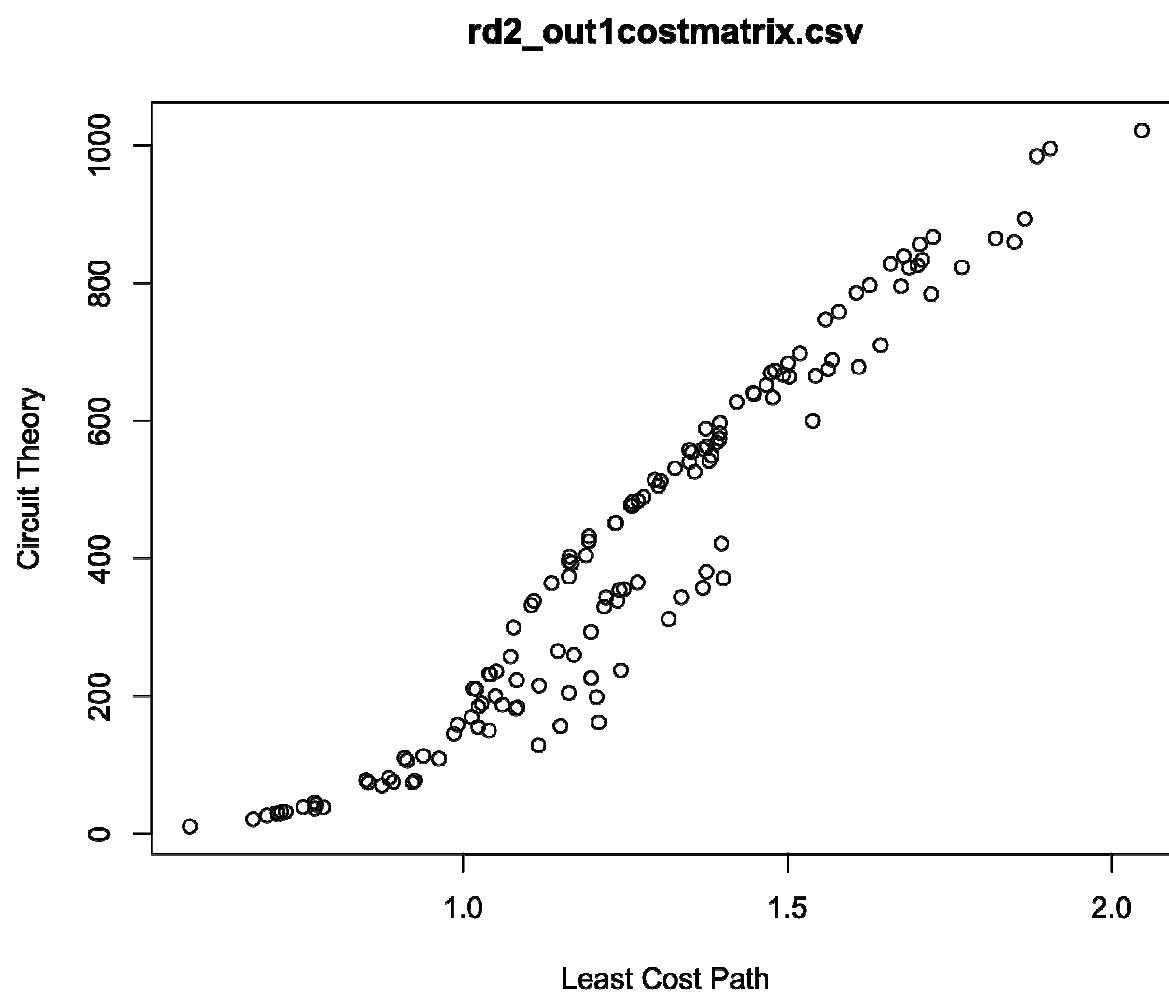


Figure S7.

Vita

Crystal Anne Meadows was born on September 16, 1983 in Henrico County, Virginia, and is an American citizen. She graduated from Mills E. Godwin High School, Richmond, Virginia in 2001. She received her Bachelor of Science with a double major in Forensic Science and Biology and a minor in Chemistry from Virginia Commonwealth University, Richmond, Virginia in 2008. She worked in Dr. Rodney J. Dyer's laboratory from 2006 to 2008 performing lab work associated with the NSF funded research grant *The Evolution of Genetic Structure in Species-Specific Plant-Insect Relationships: The Relative Importance of Biogeographical and Co-evolutionary Processes*. DEB-0543102. She was awarded a Rice Center Research Grant in 2009 for this Masters Thesis.