POPULATION GENETIC STRUCTURE, POLLEN DISPERSAL, AND LOCAL ADAPTATION IN *QUERCUS OLEOIDES* FORESTS OF COSTA RICA

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Abstract

Recent and ongoing anthropogenic land use has altered natural landscapes and resulted in isolated patches of native vegetation across the globe. This process of habitat fragmentation reduces continuous habitat into small remnants in a matrix of altered terrain. The impetus for this research was to contribute to the growing body of work on the effects of habitat fragmentation while simultaneously gaining a better understanding of the specific role that recent fragmentation played in the evolution and demography of the most ubiquitous species in one particular region. My goal was to understand the evolutionary history of *Quercus oleoides* in Costa Rica in order to more effectively conserve and possibly restore the region's seasonally dry forest in the future. How has the conversion of the seasonally dry forest of Costa Rica to an agricultural mosaic affected *Quercus oleoides* (live oak), the dominant tree species of remnant forest fragments? Although studies addressing the genetic consequences of habitat fragmentation are becoming more common, assessments of genetic structure and population viability that inform management decisions for conservation and restoration are rare. This study combined analyses of genetic diversity, pollen dispersal, and the growth and survival of various seedling families to provide an integrated evaluation of the response of a critical dry forest species to fragmentation and will help guide management and restoration efforts in the Aréa de Conservación Guanacaste (ACG).

The *Q. oleoides* forests of Guanacaste province, Costa Rica are something of a biological enigma: they are geographically disjunct and genetically distinct from conspecifics and similar species, and geographically quite restricted within Costa Rica while spanning a broad range of environments and associations within that range. *Quercus oleoides* is ectomycorrhizal in a habitat dominated by endomycorrhizal associations, possesses an atypical developmental process with regard to germination and emergence system, produces a fruit type that is extremely rare in the tropics, is wind pollinated in a habitat dominated by insect-pollinated species, is evergreen in a habitat where most species are deciduous or semi-deciduous, and its reproductive phenology is largely mismatched to the seasonally dry environment of Guanacaste, producing large crops of dessication-susceptible acorns at the beginning of a dry season more severe than what the species encounters anywhere else in its range.

Despite this seeming mismatch between traits and environment, *Q. oleoides* is by far the most common large tree wherever it occurs. As such it is an extremely important structural species in Guanacaste dry forest. Its seeds are consumed by a wide range of mammalian and avian seed predators and its evergreen habit undoubtedly has a large effect on the abiotic environment experienced by many dry forest organisms.

The subsequent chapters describe three previously unanswered questions about the past, present, and future status of *Q. oleoides* in the ACG. In Chapter 1, I characterized the standing genetic diversity of 13 *Q. oleoides* populations

and the geographic structuring of that diversity. The pattern of that diversity was compared to geographic distance, flowering time similarity, and environmental similarity among populations. The structuring of genetic diversity was also compared between two age cohorts representing pre-fragmentation individuals and post-fragmentation individuals.

I found that *Q. oleoides* in Costa Rica contained a high level of genetic diversity as well as genetic variation that is geographically structured across the landscape. The degree to which this structuring is due to fragmentation, however, is small in comparison to the genetic structure that has existed prior to fragmentation. This is somewhat counterintuitive due to the expectations provided from population genetic theory that can be applied to fragmented landscapes. If habitat fragments are isolated from one another such that gene flow no longer occurs among them, inbreeding may reduce offspring fitness and limit the viability of populations in those fragments. Isolated habitat fragments then become genetically differentiated over time due to the random process of genetic drift. Genetic diversity may also be affected because the amount of genetic variability in a population decreases due to the loss of rare alleles when the individuals carrying them are removed. This is termed a genetic bottleneck because the genetic variability of future generations is contained in the few surviving individuals. Small populations are vulnerable to stochastic environmental and demographic occurrences because adaptation by an organism to a changing environment depends on the genetic variability present in

the population. The loss of genetic diversity reduces future evolutionary options and can lead to extinction.

Population genetic variation consists of the sum of all genetic variation among individuals within the population. It can be measured by parameters including allelic richness (A) and expected heterozygosity (H_e). Allelic richness is the average number of alleles per locus and observed heterozygosity is compared to expected heterozygosity under Hardy-Weinberg equilibrium conditions. Wright's F-statistics are means of describing how genetic diversity is partitioned in a population. High values for F_{ST} indicate that subpopulations have very different gene frequencies than the total population. A loss in heterozygosity can occur with inbreeding due to the higher chance that offspring of a mating event between two individuals with the same common ancestor may share the same alleles. One method for quantifying genetic variation within species is to assay highly variable regions of repeated DNA units called microsatellites. Individuals of a population were characterized by the differences in length of 11 of these non-coding genetic units.

Although I observed no significant correlations between genetic distance and geographic distance, flowering time similarity, or environmental similarity in Chapter 1; I analyzed pollen dispersal more rigorously in Chapter 2 in order to better calculate contemporary pollen dispersal distance estimates. It is not unusual for studies of plant populations in fragmented landscapes to report few of the negative consequences predicted by theory, and that is because pollen

may actually disperse father in fragmented landscapes. My results from two separate molecular analyses of pollen dispersal distance using 8 of the microsatellite markers from Chapter 1, however, indicated that the average pollen dispersal that resulted in viable offspring predominately occurred over very short distances. Both the paternity exclusion and two-generation methods yielded similarly short dispersal distance estimates. Evidence from the physical trapping of pollen in one location indicated that pollen was capable of moving much farther, however, so the importance of long distance pollen dispersal may rely more on phenology. I observed staminate and pistillate flowering times in 10 sites over two years, but the lack of strong seasonality in flowering obscured any obvious patterns.

The geographic structuring of genetic diversity and the short average pollen dispersal distance provide a sound foundation for testing for local adaptation in *Q. oleoides* populations. In Chapter 3, I compared the growth and survival of upland and lowland maternal families in their native and foreign environments. The native environment of the populations of families differs most notably in their elevations and the lack of precipitation during the 4-5 month dry season in the lowlands. Seedlings planted in the lowland garden from both populations experienced a much higher level of mortality than seedlings planted in the upland garden, but using the aster models approach for comparing the likelihood of various models of combined growth and survival data, we did not identify evidence for local adaptation.

Overall, these experiments indicate that contemporary *Q. oleoides* in Costa Rica have a rich and complicated population genetic history that despite obvious and extensive habitat fragmentation has not severely affected genetic variation or demographic processes. The long term outlook for the recovery of the tropical dry forests in general and the *Q. oleoides* stands, in particular, is good. Little direct action by managers is required and any active planting efforts do not seem to be encumbered by site-specific seed requirements. I do recommend local seed sources, however, out of an abundance of caution. These results not only add to the field fragmentation studies by examining a common, tropical tree over multiple habitats; this work also provides applicable information to an actively managed region that is in a transitory successional state.

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CHAPTER 1

THE SPATIAL GENETIC STRUCTURE OF *QUERCUS OLEOIDES* POPULATIONS IN GUANACASTE, COSTA RICA

Understanding the processes involved in shaping population genetic structure is crucial to preserving rare and threatened species and to predicting species' responses to changing environments. In the Guanacaste province of northwestern Costa Rica, tropical live oak (*Quercus oleoides*) occurs as a geographically disjunct population at the southern range limit of the species. There it forms monodominant stands that extend over considerable environmental heterogeneity. The range of *Q. oleoides* in Costa Rica is primarily contained within the Área de Conservación Guanacaste (ACG). Prior to the establishment of the ACG the landscape had been severely fragmented through conversion of the dry forest to cattle pastures, and our results indicate a genetic bottleneck resulted from these landscape changes. At 13 sites spanning extremely dry lowlands to less seasonally variable uplands, we surveyed 11 microsatellite loci from 20 individuals: ten from a pre-fragmentation cohort (>50cm dbh) and ten from a post-fragmentation cohort (<20 cm tall). Significant genetic variability existed among the sites (AMOVA; F_{ST}=0.1, p=0.01 & R_{ST}=0.15, p<0.001), and genetic diversity is known to be higher within Costa Rica than in any other region of the species range. Bayesian clustering (STRUCTURE) identified five distinct groups in the pre-fragmentation cohort. No significant variability was found between cohorts (F_{ST}=0.002, R_{ST}=0.0) but the post-fragmentation cohort contained significantly fewer private alleles and Bayesian clustering identified just four distinct groups, indicating a possible increase in

gene flow post fragmentation. Isolation by distance explained variation at a local scale, but did not entirely explain population genetic structure at the landscape level. Environmental variation among sites was highly associated with landscape scale genetic structure, but this result was driven by only one locus. When this locus was removed from the analysis, no significant correlation was observed indicating that is likely in linkage disequilibrium with part of the genome that is under environmental selection. The extreme seasonality of precipitation in the lowlands appeared to contribute to seasonality in staminate flowering, although isolation by time, based on two years of observations in 11 stands, did not significantly explain the observed population genetic structure. Future studies should examine whether long-term trends in timing of flower production may limit gene flow between these environments and contribute to the observed population genetic structure. Complex historical processes may ultimately explain the high genetic variation of *Q. oleoides* in Costa Rica and current population genetic structure. Nevertheless, recent human disturbance also appears to have left its mark on population genetic structure in these regenerating forests.

Introduction

Identifying the mechanisms that underlie population genetic structure is critical for understanding the rate and process of evolutionary change. Understanding spatial structure of genetic diversity is also essential for plant conservation given the extent of athropogenically altered landscapes on the planet (Escudero *et al.* 2003). Moreover, knowledge of geographic patterns of genetic diversity and locally adapted genotypes can be important in predicting the response to changing environments (McClean *et al.* 2005)

Genetic structure among plant populations can arise due to processes that influence gene flow patterns such as founder events, inbreeding, isolation by distance, isolation by time, and complex historical processes that include repeated population expansion, contraction and migration (Heywood 1991). Isolation by distance (IBD) (Wright 1943) is observed when gene flow between populations declines with increasing spatial distance, and the subsequent genetic drift causes genetic differentiation among populations. Pollen and seed dispersal curves usually exhibit a leptokurtic pattern where most pollen movement and seedling establishment occurs near the parent plant (Tonsor 1985). When significant genetic structure is observed within the physical limits of gamete and progeny dispersal, mechanisms besides IBD must be shaping the observed pattern.

Differences in the timing of reproduction among populations represent another important mechanism that can limit gene flow and lead to spatially

structured genetic variation. Isolation by time (IBT) has been increasingly recognized as a potential driver of diversification and population structure (Hendry & Day 2005). In flowering plants, variation in the environmental cues for flowering may cause populations to become genetically distinct through assortative mating. For example, in *Mimulus guttatus*, divergent selection acts on flowering time in populations that differ in soil moisture, such that multiple phenotypes persist within the species (Hall & Willis 2006). Variation in the timing of snowmelt also leads to asynchronous flowering and isolation by time in many alpine species (Hirao & Kudo 2004; Yamagishi *et al.* 2005).

In addition to IBD and IBT, environmental heterogeneity and microhabitat variation have been shown to cause spatial genetic structure through selection and local adaptation (Linhart & Grant 1996). The field of "landscape genetics" utilizes highly polymorphic neutral genetic markers to assess the effects of environmental variation on population genetics (Manel *et al.* 2003; Storfer *et al.* 2007). Neutral markers have been used as indicators of the influence of abiotic factors (elevation, aspect, soil type, temperature, precipitation, etc.) on the spatial distribution of genetic variation across many taxa (Gram & Sork 2001; Volis *et al.* 2004; Hamrick & Godt 1996). The association of variation in neutral markers with certain habitat characteristics does not imply that those markers are under selection, but they may be associated with regions of the genome or genotypes that are favored in a particular habitat. Associations between genetic structure

and environmental factors may also arise through founder events or limited dispersal.

Forest fragmentation, or the conversion of contiguous forest into many small and disconnected forest patches, results in reduced habitat area, fewer individuals, and greater distance between remaining individuals. The theoretical population level responses to these results have been well described (Ellstrand & Elam 1993). Reduced genetic variation, increased inbreeding, and even extinction are predicted. Numerous studies of fragmented populations have tested for these hypothesized outcomes. For example, various plant species show significant losses of allelic diversity (e.g. Van Rossum *et al.* 2004; White *et al.* 1999; Hall *et al.* 1996), increased inbreeding (Rossetto *et al.* 2004), reduced gene flow (O'Connell *et al.* 2006; Bacles *et al.* 2004), or reduced seed production with fragmentation (Kolb 2005; Van Rossum *et al.* 2002). However, often the results in fragmentation studies do not conform to theoretical expectations (Lowe *et al.* 2005) and observe none of these detrimental consequences (discussed in Hamrick 2004). The rarity or commonness of the species does not seem to influence the likelihood of negative genetic consequences from fragmentation (Van Rossum *et al.* 2002; Honnay & Jacquemyn 2007).

If historical processes caused genetic structure prior to recent human disturbance, attempts to infer causality of contemporary genetic structure to fragmentation can be difficult (Van Rossum & Triest 2006; Llorens *et al.* 2004; Lira *et al.* 2003; England *et al.* 2003). In addition, the amount of time since the

fragmentation began may not have been long enough to observe the predicted changes caused by genetic drift (Galeuchet *et al.* 2005). Experimental approaches that apply fragmentation treatments have provided empirical results for some species (Zartman *et al.* 2006; Debinski & Holt 2000) but this approach is not practical in many long-lived perennials. In the absence of experimental manipulation, age classes have been analyzed in studies attempting to find differences in genetic diversity or genetic structure between pre-fragmentation and post-fragmentation cohorts in the pursuit of quantifying how landscape disturbance has affected particular species (Collevatti *et al.* 2001; Aldrich *et al.* 1998).

This study focuses on a geographically disjunct region of *Quercus oleoides* in Northwestern Costa Rica, the southern range limit of this widespread tropical live oak that extends to the Atlantic coast of northern Mexico. The goals of this study were 1) to investigate the potential drivers of population genetic structure at the landscape scale, and 2) to determine whether effects of fragmentation could be discerned by comparing genetic diversity and structure of pre- and post-fragmentation cohorts. We assayed 11 microsatellite loci in a total of 260 tropical live oak (*Quercus oleoides*) seedlings (<20 cm tall) and mature trees (>50 cm dbh) from 13 sites in Guanacaste, Costa Rica. The presence of staminate flowers on 11 trees at each site was monitored monthly, and the local environment at each site was characterized from existing classification maps and our own measurements of temperature, relative humidity, soil moisture, and solar

insolation. We used these data to determine support for the following hypotheses: 1) Isolation by distance due to limitations in gene flow drives spatial genetic structure; 2) Among site variability in flowering time causes genetic differentiation among sites; 3) Limits to gene flow among sites is associated with local environmental heterogeneity (linked to dry season severity). To determine whether fragmentation has influenced genetic diversity and spatial genetic structure in Costa Rican tropical live oak forests we examined if: 1) post-fragmentation generations have reduced genetic diversity and 2) spatial genetic structure differs between pre- and post-fragmentation cohorts.

Methods

Research Site

This study was conducted in the tropical dry forests of the 120,000-hectare Área de Conservación Guanacaste (ACG) in northwestern Costa Rica (Figure 1.1A). Tropical dry forest is considered one of the most threatened tropical ecosystems because of its susceptibility to fire and relative ease of conversion to agricultural development (Janzen 1988). Prior to the establishment of this conservation region in 1971, decades of fragmentation occurred that transformed thousands of acres of dry forest into cattle pastures. Over the past 37 years parcels of land have been added to the ACG, resulting in a mosaic of live oak forest and pasture that is no longer under agricultural management.

Study Species

The range of *Quercus oleoides* Cham. and Schlect. extends from the Atlantic coasts of Mexico, Belize, and Honduras to northwestern Costa Rica where it reaches its southern range limit (Montoya Maquin 1966). The Guanacaste population is disjunct from the rest of the species range and is physiologically and genetically distinct (Cavender-Bares *et al.* in review). In the seasonally dry forest of Guanacaste, *Q*. *oleoides* is the most commonly encountered tree species and it occurs over a wide range of abiotic conditions that vary with elevation. Compared to low elevation populations, higher elevation populations encounter a weaker dry season, manifested by higher soil moisture and relative humidity but lower temperatures and amount of insolation. The species forms high density and open stands from sea level to about 800 meters and usually occurs on poor quality soils. Its evergreen habit is thought to be crucial in maintaining a hospitable understory microclimate for numerous organisms during the four to five month dry season. The species is monoecious, wind pollinated, and displays considerable variability in flowering phenology. This is unique for dry forest species, since most exhibit a strong seasonal flowering flush in the dry or rainy season (Frankie *et al.* 1974). Mammals disperse acorns when *Q. oleoides* occurs in high density, or they may act mainly as seed predators in forests where live oaks occur sparsely (Boucher 1981). In Guanacaste province, flowering time among populations of *Q. oleoides* is highly variable. Herbarium specimens from Costa Rica show flowering in seven

different months. This differs from more northerly observations where phenology is more synchronous over a shorter period, similar to temperate species (Boucher 1983). *Quercus oleoides* is considered a critical species to the restoration and conservation efforts of managers of the Área de Conservación Guanacaste (Klemens *et al.* in press).

Sampling

We collected leaf tissue from 20 individual trees each from 13 sampling sites (Figure 1.1B or 1.1C). These sites represent the geographic and environmental range of *Q. oleoides* in Guanacaste. Each sampled tree was marked and its location was recorded with a GPS. Duplicate sampling of vegetative clones was avoided by sampling non-neighboring trees. Young leaves were preferentially collected and stored in a -20° C freezer for up to two weeks until the beginning of the DNA extraction process. DNA was extracted using Qiagen DNeasy Plant Mini Kit (Qiagen 2004) at Santa Rosa National Park, and the extracted DNA was amplified and analyzed at the University of Minnesota. DNA was quantified using an UV/Vis spectrophotometer and diluted with distilled water to a concentration of approximately 10 ηg/mL for polymerase chain reaction (PCR) amplification.

Microsatellite Data Collection

Nine of the eleven microsatellites employed (*ZAG15, ZAG110, ZAG 9, ZAG 1/2, ZAG 1/5, ZAG 102, ZAG 36, ZAG 16, ZAG 46*) were developed for *Quercus petraea* by Steinkeller *et al*. (1997) The other two (*ZAG 30 and ZAG 11*) were developed by Kampfer *et al*. for *Quercus robur* (Kampfer *et al.* 1998). A multiplex PCR approach was taken where each PCR reaction included 3 or 4 primer pairs (Qiagen 2007). 10 μl PCR reactions consisted of 1.0 μl of the fluorescently labeled forward primer and 1.0 μl of the reverse, 5.0 μl Qiagen Master Mix, 1 μl (~10 ηg) DNA, and sterilized water. The thermal cycles for this reaction were: 94°C for 15 min, followed by 35 cycle s of 30 s at 94°C, 30 s at 50°C and 60 s at 72°C. A final extension step of 1 0 min at 72°C was added after the last cycle. An ABI 377 Automated Sequencer at the University of Minnesota's Advanced Genetics Analysis Center was used to measure the length of the fluorescently labeled PCR product. We used the program GenoProfiler to characterize alleles by the length of the amplified fragments (You *et al.* 2004). Petit *et al*. (2002) found a region of the chloroplast genome that provided enough variation to differentiate between oak species in Europe using restriction fragments, but these trnD and trnT markers showed no variation in samples of *Q. oleoides* from Guanacaste.

Analysis of Molecular Data

In the original publications of these microsatellite markers, the authors found them all to fit neutral expectations. We confirmed this using an Ewens-Watterson test in the program PopGene (Yeh & Boyle 1997). We calculated the number of alleles per locus (A), percentage of polymorphic loci (P), observed heterozygosity (H_O), Nei's expected heterozygosity (H_E) (Nei 1973a) and a fixation index (F) for all individuals combined and separated into cohorts. These measures indicate levels of genetic diversity and allow for comparisons among populations. Genetic differentiation among populations was determined by the infinite allele model F_{ST} and analysis of molecular variance (AMOVA) (Weir & Cockerham 1984). Among population differentiation was also quantified by R_{ST} using a stepwise-mutation model. Differences between FST and RST can indicate the relative importance of drift and mutation in causing the observed differentiation (Hardy *et al.* 2003). We use the term populations to refer to each of the 13 sampling sites. The degree of genetic similarity between populations was based on Nei's genetic distance (Nei 1973b). We also calculated a rough estimate of geneflow according to the Slatkin and Barton method ($N_m = 0.25(1-F_{ST}/F_{ST})$ (1989) in PopGene (Yeh & Boyle 1997), but this type indirect measurement has been shown to be a poor estimator of contemporary gene flow in disturbed landscapes (Whitlock & McCauley 1999).

We tested for IBD using a Mantel test, which quantifies the correlation between Nei's genetic distance and geographic distance (Smouse *et al.* 1986). Geographic distance was obtained using GPS coordinates in the center of each sampling site. Significance was determined by comparing the observed correlation to random permutations of the data. IBD would be indicated by a more positive correlation than expected. Additionally, we calculated a multivariate spatial autocorrelation coefficient following Smouse and Peakall (1999). The autocorrelation coefficient, *r* (-1 to 1), is similar to Moran's I (Moran 1950) where -1 would indicate perfect genetic dissimilarity and +1 genetic identity. It was used to calculate genetic similarity between pairs of individuals separated by 7 km distance class intervals. 7km intervals were chosen by gradually increasing the class interval until positive genetic structure was no longer detected, per the authors' suggestion. To test for statistical significance, a null distribution was made by random permutation of all individuals across the distance classes 1000 times. A confidence estimate around the observed *r* was drawn from 1000 bootstrap trials (with replacement) from within each distance class. All above analyses were conducted using GenAlEx (Peakall & Smouse 2006) and Arlequin (Excoffier *et al.* 2001).

The number of genetically distinct clusters of all individuals and individuals in each cohort was estimated using a Bayesian approach in which a Markov Chain Monte Carlo method clustered individuals to minimize Hardy-Weinberg disequilibrium and linkage disequilibrium (STRUCTURE, Pritchard *et al.* 2000).

This method does not assume a particular mutation model and probabilistically assigns individuals into K populations, or multiple populations if there is admixture. Three runs were performed for each K (putative number of distinct genetic clusters) and the range of possible K's tested was from 1 to 13 because of the 13 sampling sites. The USEPOPINFO flag was set to 0, commanding the program not to take the sampling location into account in assigning clusters. Runs were performed with a burn-in length of 50,000 and a MCMC of 1,000,000. The correct K was selected by observing the highest log likelihood values (ln Pr[X|K]) averaged over the three runs. Evanno *et al*. (Evanno *et al.* 2005) detect the correct K by comparing the magnitude of the change in log likelihood value (delta K) because of problems with increasing log likelihood scores with increasing K's. We compared delta K values for all individuals and the two cohorts separately.

Environmental Data Collection

We described the environment of each site using elevation, the Holdridge life zone classification system, and a climate classification specific to Costa Rica. The Holdridge system defines life zones based on annual precipitation and biotemperature (Σ mean monthly temperature >0℃ /12) (Holdridge 1947). Because the seasonal fluctuation in precipitation is obscured by annual data, we included the climate classification from Herrera that quantifies the effect of the dry season (Herrera 1986). It comprises an aridity index (degree of water deficit

below water need) and a hydric index (amount of water available for plants). These measures are commonly used in biogeography studies because of strong association with vegetation (Becker *et al.* 1994). GIS layers of these classifications for the ACG are shown in Figures 1.1B and 1.1C. Finally, we included elevation as a vector in the environmental description because it is related to soil water availability, temperature, precipitation, and solar insolation.

The life zone and climate classifications at each of the 13 sampling sites were treated as ordinal variables that increase with temperature, precipitation, and elevation (Table 1.1). Normalized Euclidean distances among the sites were calculated from elevation, life zone, and climate type to make a matrix of environmental dissimilarity using the program Primer (Clarke & Gorley 2001). This provides an integrated measure of all the relevant environmental variation.

We chose this method over data interpolation methods like WorldClim (Hijmans 2005), where worldwide weather station data is interpolated across areas where no local data is available. That method identified few differences among our sites because the spatial resolution was too coarse, as has happened in other studies that found this method unreliable (Daly 2006). We believe that the chosen environmental classifications are the best substitute for detailed meteorological measurement at each site because they incorporate geological, climatic and biologic information in characterizing the local environment.

At a subset of six sites (Table 1.1) we were able to collect detailed climatic data. Monthly soil moisture was recorded using time domain reflectometry

(TDR), following previously described methods (Cavender-Bares & Holbrook 2001). Daily photon flux density (PFD) was measured using gallium arsenide light sensors (Hamamatsu Photonics) following established procedures (Givnish *et al.* 2004) 50 cm above the forest floor and 20 m in from the forest edge. Campbell Scientific 21X and Hobo dataloggers recorded the PFD, as well as temperature (T) and relative humidity (Rh) every 10 minutes over a two-week period during the wet and dry seasons in two consecutive years. We created an environmental distance matrix from these data by calculating the normalized Euclidean distance among the six sites using the same method as above.

Phenological Data Collection

At 10 of the 13 sites we made phenological observations on one focal tree and its ten nearest neighbors. We recorded the presence of male flowers once per month for two years. It was not possible to visit the other three sites (EH, LP, PSE) every month due to poor accessibility. Similarity of staminate flowering time among sampling sites was calculated according to Schoener's index of similarity (Schoener 1970) to describe the proportion of trees that were flowering synchronously.

$$Similarity_{ih} = 1 - 0.5 \times \sum \left| p_{ij} - p_{hj} \right| \qquad \text{eq. 1}$$

The similarity of sites *i* and *h* at time *j* depend on the proportion of trees that were flowering (*p*) (eq. 1). Values were calculated using a Visual Basic program described in Cavender-Bares *et al*. (2004).

We also used an alternate calculation to quantify the degree of overlap in flowering times based on Pianka's index of niche overlap using EcoSim (Gotelli & Entsminger 2001).

$$O_{jk} = O_{kj} = \frac{\sum_i^n E_{ij} E_{ik}}{\sqrt{\sum_i^n \left(E_{ij}^{\ 2}\right) \sum_i^n \left(E_{ik}^{\ 2}\right)}} \qquad \text{eq. 2}$$

Overlap (O) between sites *j* and *k* depends on the proportion of individuals with staminate flowers (E) in sites *j* and *k* at time *i* (eq. 2).

Genotype–Environment–Phenology Analysis

Correlations between genotype and the environment, and genotype and phenology were tested by matrix correlation analyses just as in the test for IBD above. To calculate significance values, 1000 random permutations of the rows and corresponding columns of one of the matrices were made to construct the reference distribution which was compared to the observed distribution. A significant effect of environmental dissimilarity on genetic dissimilarity would be indicated by a more positive correlation than expected. A test for the association of flowering time with genetic distance from the two flowering time calculations

above would be indicated by a negative correlation because they are based on similarity rather than distance.

Partial Mantel tests, where the correlation between two matrices are tested while removing the effect of a third matrix, were calculated for all interactions in the program ZT (Bonnet & Van der Peer 2002). The test works by first constructing a matrix of residuals from the simple linear regression between the distances from the first matrix and the distances in the third matrix. Then a second matrix of residuals is made from a regression of the second distance matrix and the third. The two residual matrices are then compared by a standard Mantel test. This allowed us to test the correlation between genetic and environmental distance while partitioning out the effect of spatial distance. Similarly, we tested the correlation between the genetic distance and flowering phenology while omitting any spurious correlation with spatial distance or environmental distance. There is debate about the accuracy of type I error probabilities for partial Mantel tests (bias due to observed type I error in simulations at intended 0.05 level from 0.000-0.092 depending on variance values) (Rousset 2002), but the test is still regarded as an important means of evaluating residual effects when two matrices are correlated (Storfer *et al.* 2007).

Results

Population Genetic Variation

A total of 257 individuals (128 mature trees, 129 seedlings) were successfully assayed at 11 microsatellite loci. Allelic diversity was high with each locus containing between 6 and 17 alleles (Table 1.2). Each site averaged at least four alleles per locus and 91% - 100% of the loci were polymorphic per site (Table 1.2). Most of the loci showed a slight excess of heterozygotes and little evidence of inbreeding (Table 1.2). There was no evidence of genetic differentiation between pre-fragmentation and post-fragmentation generations (F_{ST}=0.002, R_{ST}=0.0), and an AMOVA found that 0% of variance was explained by the two age cohorts. The cohorts have similar expected heterozygosities, but the post-fragmentation generation has fewer private alleles (Figure 1.2).

The Ewen's-Watterson test for neutrality did not indicate non-neutral behavior for any of the loci, but a fundamentally different pattern was occurring in one marker, ZAG 15. It appears to be in linkage disequilibrium with a region of the genome that is affected by some or all of the factors in the environmental distance matrix. We are unsure what is driving the observations but it is clearly a different process than the rest of the markers. For all genetic analyses, we include a result with ZAG 15 as well as a result excluding this marker when the two results differ.

Significant structure was found among the 13 sampled populations based on AMOVA (F_{ST} = 0.102, p=0.01 & R_{ST}=0.15, p<0.001; Table 1.3). An UPGMA

tree (PAUP, Swofford 2003) based on Nei's distances among sites indicates that some sites that are proximate spatially are genetically similar (SE and CF or VJ and AO) but the genetically similar sites in the bottom clade (LP, SJ, PSE and RM) are separated by as much as 65 km (Figure 1.3A). The indirect measurement of gene flow from F_{ST} did not indicate much difference between cohorts (N_m Adults = 1.54 and N_m Seedlings = 1.55).

The Bayesian structure analysis revealed 5 distinct genetic clusters (ln Pr[X|K] = -6970 for K=4, -6875 for K=5, and -6972 for K=6; Table 1.4 and Figure 1.4A**).** This number of clusters remained the same when ZAG 15 was excluded (Figure 1.4B). The observed log likelihood scores here provide a clear indication of the most likely number of clusters because the values clearly peak, but results from the delta K method conflicted with these K's somewhat. For all seedlings and adults combined, the delta K value for K=2 (13.26) was slightly higher than for K=5(11.24). When the cohorts were analyzed separately, 5 populations were again indicated for the adults (Table 1.4 and Figure 1.5A) whereas only 4 seedling populations were indicated by the log likelihood score (ln Pr[X|K]= -3569 for K=3, -3512 for K=4, and –3529 for K=5; Table 1.4 and Figure 1.5B). The difference between these average log likelihood scores (ln Pr[X|K]= -3566.3 for 5 adult clusters and ln Pr[X|K]= -3512 for 4 seedlings clusters) was significant according to a t-Test (p=0.006). The delta K values for seedlings closely followed the log likelihood interpretation (K=2 = 4.73 and K=4 = 4.48). UPGMA trees of each cohort also show different groupings of populations for the two

cohorts, primarily with the placement of SC, an upland population, with the other upland populations (VJ and AO) post-fragmentation but not pre-fragmentation (Figure 1.3B & 1.3C).

This structure could not be accounted for by isolation by distance according to a Mantel test ($r=0.17$, $p=0.14$). The multivariate spatial autocorrelation analysis illustrated that individuals that were less than 14 km apart had significant genetic similarities, but so did those that were about 49 km and 70 km apart (Figure 1.6). Pre-fragmentation individuals began to become more different from one another at shorter distances than seedlings, and incongruence between the spatial structure of the age cohorts are apparent at 14, 35, and 49 kilometers where one cohort's degree of similarity is significantly different from random while the other's is not. Non-overlapping error bars between the two cohorts indicate that their patterns of genetic variation with distance are significantly different.

Genotype–Environment–Phenology Analyses

Phenological observations provide evidence for a large amount of variability in staminate flowering time among the 10 observed sites (Figure 1.7). Some sites flowered as many as seven months per year, whereas others only three. The two methods for calculating phenological similarity provided nearly equal correlations with genetic and environmental distances (Appendix 1) so only results from eq. 1 are shown. There was no significant correlation between

genetic distance and flowering time among the 10 sites (r= -0.08, p=0.28 all loci; r= -0.03, p=0.40 without ZAG 15; Table 1.5). There was a trend toward a correlation between phenology and spatial distance (r= -0.19, p=0.096) and a significant correlation with environment (r= -0.28, p=0.048). The significance of this correlation was diminished in the partial Mantel test by the effect of spatial distance (r= -0.24, p=0.078). In the subset of sites where we had measured environmental data (maximum and minimum daily temperature, maximum and minimum daily relative humidity, daily PAR, and monthly soil moisture) the results mirror those where the environmental matrix is made from classification systems (Table 1.5). The low correlation for partial Mantel tests in the six sites could be due to the fact that the spatial distances between the low and high elevation sites are much greater than between the sites within each elevation. In this case we only have a few very near geographic distances and a few very far distances to compare, as opposed to the full analysis which included more intermediate distances. This makes it difficult to differentiate the effects of spatial and environmental distance.

The environmental distance matrix, calculated as the normalized Euclidean distance between each site's elevation, life zone, and climate type, was significantly correlated with the full genetic distance matrix (r=0.29, p=0.017 all loci; r=0.12, p=0.16 without ZAG 15; Table 1.5). In a locus by locus analysis, only the genetic distance matrix from locus ZAG 15 had a significant correlation with the environmental distance matrix (r=0.65, p=0.001). When this locus was

removed from the genetic distance matrix, none of the previously significant correlations were observed (in parentheses, Table 1.5). There was a significant correlation between the environmental and spatial distance matrices (r=0.31, p=0.012), but spatial distance was not significantly correlated with genetic distance (r=0.17, p=0.14 all loci; r=0.05, p=0.25 without ZAG 15). A partial Mantel test between the genetic distance and environmental distance, partitioning out the effects of spatial distance, remained significant until ZAG 15 was removed (r=0.25, p=0.052 all loci; r=0.11, p=0.21 without ZAG 15; Table 1.5).

When the age classes were analyzed separately, genetic distances among sites in the pre-fragmentation cohort and the post-fragmentation cohort were significantly correlated with environmental distance (r=0.23, p=0.045 and r=0.29, p=0.016 respectively; Table 1.6). When accounting for the geographic distance in a partial Mantel test, however, the significant correlation remained between the genetic distance and environmental distance matrices of the pre-fragmentation cohort (r=0.24, p=0.047), but that correlation is weaker and only marginally significant in the post-fragmentation cohort (r=0.19, p=0.082; Table 1.6). This is probably due to the much higher correlation between the genetic distance and geographic distance in the seedlings (r=0.23) than the pre-fragmentation cohort (r=0.06). Removing ZAG 15 from the genetic distance matrix caused the correlations to no longer be statistically significant (Table 1.6). Matrices of pairwise genetic distance, spatial distance, environmental distance, and phenology similarity matrices between sites are provided in Appendix 1.

Discussion

Our study of the population genetic structure and diversity of a widespread and locally abundant tropical oak, *Quercus oleoides*, reveals high genetic diversity and significant genetic subdivision. This is in contrast to other studies of common, widespread species (Hamrick & Godt 1996). These results indicate that population genetic structure cannot easily be predicted based on particular distribution and abundance characteristics. In Costa Rica, Q. oleoides forms a geographically disjunct population group that has higher genetic diversity than in any other region (Cavender-Bares *et al.* in review). Here, populations at various spatial distances are sometimes more and sometimes less likely to be genetically similar than expected by chance alone, indicating that historical and/or ongoing beyond physical separation are preventing gene flow adequate for panmixia. Local environmental variation and differences in flowering time likely play a role, but we cannot directly attribute the observed genetic structure to our data on these factors. Our analysis of the effects of forest clearing on the existing population structure shows reduced population genetic structure of the post-fragmentation generation relative to pre-fragmentation generation.

Significant among population genetic differentiation

In contrast to studies of population genetic structure in abundant and widespread temperate oak species of North America (Craft & Ashley 2007; Craft

& Ashley 2006; Dow & Ashley 1998; Dow & Ashley 1996), we found significant differentiation among local populations of *Q. oleoides* in northwest Costa Rica. In this study, eleven microsatellite loci were assayed in 157 individuals and were found to be highly polymorphic with an average of 3-6 alleles per locus across sites (Table 1.2). The 13 populations had a statistically significant level of among population genetic variation (Table 1.3), and Bayesian clustering found 5 likely genetically distinct clusters of individuals (Figure 1.4). Differentiation among populations under a stepwise-mutation model, as measured by R_{ST}, showed slightly higher values, indicating that stepwise-like mutation contribute to population differentiation (Hardy *et al.* 2003).

We found no variation in chloroplast markers in the TrnD/TrnT region, contrasting other studies of oak populations in temperate regions of Europe (Petit *et al.* 2002b; Petit *et al.* 2002a; DumolinLapegue *et al.* 1997). The lack of chloroplast variation may be the result of historically small population sizes, and long-term isolation that led to fixation of a single choloroplast haplotype (Cavender-Bares *et al.* in review). Recent population expansion post-glaciation is a common explanation for the lack of population genetic structure in North American oak populations examined at regional (subcontinental) scales. The significant population structure found using chloroplast haplotypes in post-glacial populations in Europe are found only at continental scales and have been interpreted as the result of isolation between glacial refugia (Petit *et al.* 2002a). In the tropics, the lack of glacial cycles may have allowed a longer period for

population genetic structure to develop (Grivet *et al.* 2006), even at regional scales, such as Costa Rica.

Population genetic structure and the environment

There is considerable heterogeneity in climate in northwestern Costa Rica, with highly seasonal precipitation at low elevations and a much less severe dry season at high elevations. Such heterogeneity could influence reproductive phenology, given that flowering requires hydraulic support, with consequences for pollen distribution and population genetic structure. Isolation by distance (IBD) appears to influence population structure over short spatial scales but this process cannot explain how some physically distant populations are more similar than some that are physically nearby (Figure 1.5). When we compared the genetic distance matrix to an environmental distance matrix created from existing classification systems (Table 1.1), we found a significant association (Table 1.5) that was predominantly driven by one locus (ZAG 15). This locus may be under linkage disequilibrium with a region of the genome under selection. When ZAG 15 was removed from the analysis, neutral population genetic structure was no longer significantly associated with environmental heterogeneity. Genetic differentiation was strongest, however, among populations from contrasting environments. Removing ZAG 15 did not affect overall F_{ST} values or the most likely number of genetically distinct clusters in STRUCTURE, and population genetic structure persisted in the remaining 10 markers.

The amount and seasonality of rainfall are critical environmental factors that differ among populations (Table 1.1). Water availability is involved in cuing flowering (or is correlated with other factors that are) in many tropical species (Jackson 1966) Water availability can also impose limits to flower production because flowering requires significant hydraulic support to develop and sustain desiccation-sensitive tissues (Chapotin *et al.* 2003). Flowering asynchrony resulting from environmental heterogeneity is known to cause non-random mating (Loveless & Hamrick 1984) making isolation by time (IBT) a plausible factor in shaping the genetic structure in this system. Little is known about IBT in the tropics so our observations of *Q. oleoides* make it an important study system.

The variation in habitats and elevation where *Q. oleoides* occurs as well as its commonness and fairly restricted range also make this an important species in which to test this hypothesis. There was a significant correlation between phenology and environment, indicating that some of the environmental variables we measured may influence the timing of flower production. In the higher elevation sites, we observed staminate flowers on at least one tree in 22 of the 24 months that we made observations (Figure 1.7). In contrast, at lower elevations staminate flowers were only observed in the 12 wettest months, indicating more seasonality. Despite these patterns, similarity in staminate flowering time was not found to be directly associated with overall genetic structure. A possible explanation for the lack of correlation between phenology and genetic structure could be that male flowering does not provide an accurate

measure of potential gene flow. Overlap of pollen and stigma viability would better indicate potential gene flow given that the energetic cost to the tree is presumably lower in producing pollen versus seeds. Moreover, given the high level of variation in phenology, we may not have sufficient power to detect an association between flowering time and genetic structure. The flowering patterns may become more evident over many years of observations or across more stands. Future studies are necessary to detect the extent to which IBT may be operating in this system.

There are several additional potential causes of highly structured among population genetic variation. These include differential gene exchange due to asymmetrical pollen or seed dispersal, chance associations caused by genetic drift or founder effects, selection on genomic regions near the surveyed microsatellite markers (Gram & Sork 2001) and complex historical processes associated with repeated expansion, contraction, and migration of populations during dynamic periods of glacial and interglacial climate change (Pennington *et al.* 2000) and volcanic activity (Cavender-Bares *et al.* in review). While genetic drift is apparently causing some of the observed population structure, it does not adequately explain landscape level patterns. IBT is a possible cause, but the evidence for it is ambiguous in this study and we did not find a significant association between the environment-associated locus, ZAG 15, and phenological similarity. Dispersal limitation could reinforce existing population structure, and has been used to explain spatial structure in populations of other

oak species (Berg & Hamrick 1995). *Q. oleoides* seeds are dispersal-limited in this region (Klemens *et al.* in press), and results of paternity analyses in this same system (Chapter 2) suggest that effective pollen dispersal occurs over relatively short distances. Anisotropy (directionality in prevailing winds) is another potential cause of population genetic structure, and there are strong prevailing W-SW winds in this region especially during the dry season (Janzen 1983). However, an examination of anisotropic autocorrelation in *Quercus lobata* populations of California, where there are strong prevailing winds, detected no significant presence of anisotropy but rather a strong isolation by distance signal (Dutech *et al.* 2005).

Lower diversity and altered structure in postfragmentation cohort

Studies attempting to discern the population genetic effects of habitat fragmentation have found few certainties (Lowe *et al.* 2005). Population genetic theory predicts that the fragmentation should have adverse consequences for genetic diversity in the same way that small population sizes do (Young *et al.* 1996; Saunders *et al.* 1991; Templeton *et al.* 1990). The significant reduction in the number of private alleles in the postfragmentation cohort that we found supports the idea that fragmentation reduces genetic diversity. Furthermore, STRUCTURE identified one fewer genetically distinct cluster in postfragmentation individuals and the difference between the likelihood scores for K=5 adults and K=4 seedlings was statistically significant (Table 1.4). These

results support theoretical predictions that when population size is reduced, rare alleles are lost. This may be depicted by the shift in the relationship between SC and the other upland populations (VJ and AO) pre-fragmentation to post-fragmentation illustrated in the UPGMA trees (Figure 1.3B & 1.3C).

The reduction of the number of genetically distinct clusters proposed by the STRUCTURE analysis also suggests longer distance contemporary pollen dispersal than that which occurred when forests were contiguous. Likewise, the spatial autocorrelation data (Figure 1.6) corroborates this interpretation, revealing a contrasting pattern of spatial genetic structure in seedlings relative to adults. Seedlings do not show significant population differentiation until individuals separated by 35 km were compared, whereas the adults were differentiated at 14 km. Post-fragmentation, it appears as though one unique pre-fragmentation population (PS) has joined the three central populations (SE, CF, FJ) (Figures 1.5A and 1.5B). This is also one of the most heavily fragmented areas of the ACG and therefore more likely to result in more frequent long distance pollination events.

Other studies have also shown evidence for increased pollen dispersal distances across highly fragmented populations relative to intact forest stands (Dow & Ashley 1998; Robledo-Arnuncio & Austerlitz 2006; Victory *et al.* 2006; Bacles *et al.* 2005; White *et al.* 2002; Dick *et al.* 2003). In a study similar to this one that used cohorts to investigate the effects of fragmentation, Marquardt and Epperson (2004) found significant spatial autocorrelation, indicating IBD, in old

growth *Pinus strobus* but not in a second growth stand that grew up after a clear cut. In contrast to the present study, they did not find a reduction in private alleles in the post-cutting cohort. Studies in non-fragmented forests indicate that there are other explanations for differences in population structure between age or size cohorts. For example, genetic differentiation has been reported between seedlings and older cohorts in *Jacaranda copaia* in non-fragmented forests of Panama (Jones & Hubbell 2006). *Jacaranda copaia* depends on light gaps and many seedlings suffer great mortality unless a large canopy gap appears. This apparently allows closely related individuals in that gap to form the next age cohort. Unlike *J. copaia*, however, regeneration of *Q. oleoides* occurs in the understory of monodominant stands (Klemens *et al.* in press), facilitated by the relatively low-density canopy of live oaks (Boucher 1983; Spector & Putz 2006). This regeneration biology may prevent genotypes from being clustered. In a separate study in Eucalyptus forests in Australia, Jones *et al*. (2007) used spatial autocorrelation in adults and juvenile *Eucalyptus globulus* trees to show a differential effect of wind direction on spatial genetic structure in different age classes. Not all studies have found differences in population genetic structure between cohorts. In an undisturbed landscape, multiple reproductive cohorts of a perennial tree, *Neolitsea sericea* showed no difference in spatial genetic structure (Chung *et al.* 2000).

An alternative interpretation of the reduction in genetic differentiation in the post-fragmentation cohort is that certain selective filters have eliminated widely

dispersed genotypes in locally adapted populations. Current seedling population genetic structure would thus develop into a structure similar to that observed in the adult cohort after selection. Epperson and Alvarez-Buylla (1997) found strong spatial genetic structure among seedlings that declined among saplings and adults in the tropical forest gap species, *Cecropia obtusifolia*. But we observe a genetic bottleneck, a loss of diversity and reduced structure, indicating that landscape changes are involved in creating the post-fragmentation genetic structure.

Table 1.1. Environmental characterization for each site

Site	Elevation (ft)	Life Zone[ℓ]	Climate type[n]
FJ*	675	2	2
SE*	900	2	2
CF*	900	2	2
SC*	2600	5	6
VJ*	2700	4	6
AO*	2800	5	6
PS	1000	2	3
EH^	1000	2	3
LP^	1700	3	4
SJ	2000	4	5
GY	475	1	1
PSE^	1000	2	2
RM	800	2	2

**subset of six sites where environmental data were collected over two week periods in the 2005 and 2006 wet season and the 2006 dry season (average total daily PAR, average daily high and low temperature, average daily high and low relative humidity), as well as monthly soil moisture observations over two years).*

^sites not included in monthly phenological monitorring.

[ℓ]Life Zone

1	Tropical Dry Forest
2	Premontane Moist Forest Basal Transition
3	Tropical Moist Forest
4	Tropical Moist Forest Premontane Transition
5	Premontane Wet Forest

Holdridge classification from Tosi (1969).

[n]Climate Type

	Classification	Average Annual Precipitation (mm)	Average Annual Temperature (°C)	Annual Potential Evapotrans-piration(mm)	†Aridity Index (%)	‡Hydric Index (%)
1	A1	1300-1710	>27	>1710	>20	-33.3-0
2	B1	1710-2050	23-27	>1710	>20	0-20
3	B4	1565-2052	21-26	1565-1710	>20	0-20
4	C4	1900-2400	21-26	1565-1710	>20	20-40
5	D4	2200-2800	21-25	1565-1710	>20	40-60
6	E8	2300-2800	18-24	1420-1565	10-20	60-80

†aridity index: 0-10 = 0-35 days of water deficit; 10-20 = 35-70 days of water deficit; >20 = >70 and <150 days of water deficit.

*‡hydric index = ((average annual precipitation/annual potential evapotranspiration)-1) * 100.*

classification and data from Herrera (1986).

Table 1.2. Genetic diversity estimates for each site averaged over 11 microsatellite loci

Site	N	N_A	%P	H_o	H_e	F
FJ	19.4	5.8 (5.9)	100%	0.639 (0.637)	0.619 (0.621)	-0.046 (-0.042)
SE	19.7	5.4	100%	0.471 (0.493)	0.552 (0.580)	0.121 (0.125)
CF	18.8	5.2 (5.0)	100%	0.607 (0.604)	0.599 (0.595)	-0.019 (-0.022)
SC	19.0	4.4	100%	0.608 (0.595)	0.562 (0.559)	-0.084 (-0.069)
VJ	19.5	5.4 (5.3)	100%	0.634 (0.617)	0.621 (0.609)	-0.036 (-0.032)
AO	19.8	5.2 (5.3)	100%	0.577 (0.555)	0.608 (0.600)	0.034 (0.055)
PS	19.7	4.4	100%	0.543 (0.551)	0.570 (0.585)	0.027 (0.039)
EH	18.7	4 (4.1)	100%	0.506 (0.515)	0.560 (0.581)	0.079 (0.106)
LP	20.0	4.3 (4.1)	91%	0.513 (0.505)	0.521 (0.514)	0.017 (0.017)
SJ	19.5	3.8 (3.6)	91%	0.553 (0.524)	0.512 (0.496)	-0.069 (-0.042)
GY	20.0	4.7 (4.5)	100%	0.559 (0.530)	0.513 (0.487)	-0.030 (-0.022)
PSE	19.4	4.4 (4.2)	91%	0.517 (0.514)	0.495 (0.492)	-0.016 (-0.010)
RM	19.5	4 (3.9)	91%	0.520 (0.522)	0.487 (0.487)	-0.065 (-0.061)

N sample size, N_A number of alleles, P percentage polymorphic loci, H_o observed heterozygosity, H_e expected heterozygosity, F fixation index. Loci (bold) and numbers of alleles per locus (in parentheses) were as follows: **ZAG 15** (7), **ZAG 30** (11), **ZAG 110** (6), **ZAG 9** (13), **ZAG 1/2** (8), **ZAG 1/5** (7), **ZAG 102** (17), **ZAG 36** (9), **ZAG 16** (10), **ZAG 46** (9), **ZAG 11** (6). *ZAG15 omitted in parentheses*.

Table 1.3. Analysis of molecular variance (AMOVA) for 13 sampling sites

Source	df	SS	MS	Est. Var.	%
Among Pops	12	207.493	17.291	0.358	10%
Within Pops	501	1570.905	3.136	3.136	90%
Total	513	1778.399	20.427	3.494	
F_{ST} = 0.102, p=0.01					
Source	**df**	**SS**	**MS**	**Est. Var.**	**%**
Among Pops	12	35254.116	2937.843	64.953	15%
Within Pops	501	185260.863	369.782	369.782	85%
Total	513	220514.979	3307.625	434.736	
R_{ST} = 0.15, p<0.001					

Table 1.4. Average lnP(X|K) of three runs for each K from STRUCTURE

	1	2	3	4	5	6
All	-7672.0	-7297.1	-7107.6	-6969.7	*-6875.3*	-6971.7
Adults	-3849.7	-3677.4	-3613.0	-3607.3	*-3566.3*	-3583.8
Seedlings	-3841.2	-3675.7	-3568.9	*-3512.0*	-3528.9	

italics indicate highest log-liklihood

With ZAG 15 omitted for all individuals, 5 remained the most likely K

1=-6931.2, 2=-6559.7, 3=-6410.1, 4=-6297, *5=-6211.3* , 6=-6294.2

t-Test between K=5 for adults and K=4 for seedlings, p=0.006

Table 1.5. Pearson correlation coefficients from Mantel tests between matrices

	Genetic	Spatial	Environment	Phenology
Genetic				
Spatial	0.17 (0.05)			
Environment	0.29* (0.12)	0.31*		
Phenology	-0.08 (-0.04)	-0.19[1]	-0.28*	

»***Genetic-Environment-Spatial*** = 0.25* (0.11)

»***Phenology-Environment-Spatial*** = -0.24[1]

subset of 6 sites using different environmental variables

	Genetic	Spatial	Phenology
Environment	0.68* (0.57[1])	0.88	-0.46*

»***Genetic-Environment-Spatial*** = 0.25 (0.31)

»***Phenology-Genetic-Spatial*** = 0.13 (0.01)

»***Phenology-Environment-Spatial*** = 0.08

»partial Mantel tests below boxes (tested over residuals of third matrix). Spatial and Environmental are Euclidean distance matrices, Genetic distance from Nei, Phenology is a similarity matrix.

*1000 permutations, [1]p<0.1, *p<0.05*

ZAG 15 removed in parentheses

Table 1.6. Mantel test and partial Mantel test for each cohort separately

	Prefragmention		Postfragmentation	
	r	*p*	**r**	*p*
Genetic-Environmental	0.23 (0.13)	0.045* (0.18)	0.29 (0.14)	0.016* (0.14)
Genetic-Environmental-Geographic	0.24 (0.15)	0.047* (0.17)	0.19 (0.11)	0.082 (0.22)

ZAG 15 omitted in parentheses

Figure 1.1A) Map of Central America with area of study in Costa Rica enclosed in dashed box.

Figure 1.1B) Close up of area in dashed box with sampling points plotted and labeled (AO=Agave Oaks, CF=Cortafuego, EH=El Hacha, FJ=Finca Jenny, GY=Guayabo, LP=La Perla, PS=Pocosol, PSE=Peninsula Santa Elena, RM=Ranch Mari, SC=Sendero Caballo, SE=Santa Elena, SJ=San Jorge, VJ=Valle Jabely). Lines trace different forest types based on Holdridge Life Zones (1967) (map by W. Medina www.investigadoresACG.org). Key to life zone numbers in Table 1.1.

Figure 1.1C) Close up of area in dashed box with sampling points plotted and labeled (AO=Agave Oaks, CF=Cortafuego, EH=El Hacha, FJ=Finca Jenny, GY=Guayabo, LP=La Perla, PS=Pocosol, PSE=Peninsula Santa Elena, RM=Ranch Mari, SC=Sendero Caballo, SE=Santa Elena, SJ=San Jorge, VJ=Valle Jabely). Lines trace different climate zones based on Herrera's classification (1985) (map by W. Medina www.investigadoresACG.org). Key to climate type numbers in Table 1.1.

Figure 1.1A.

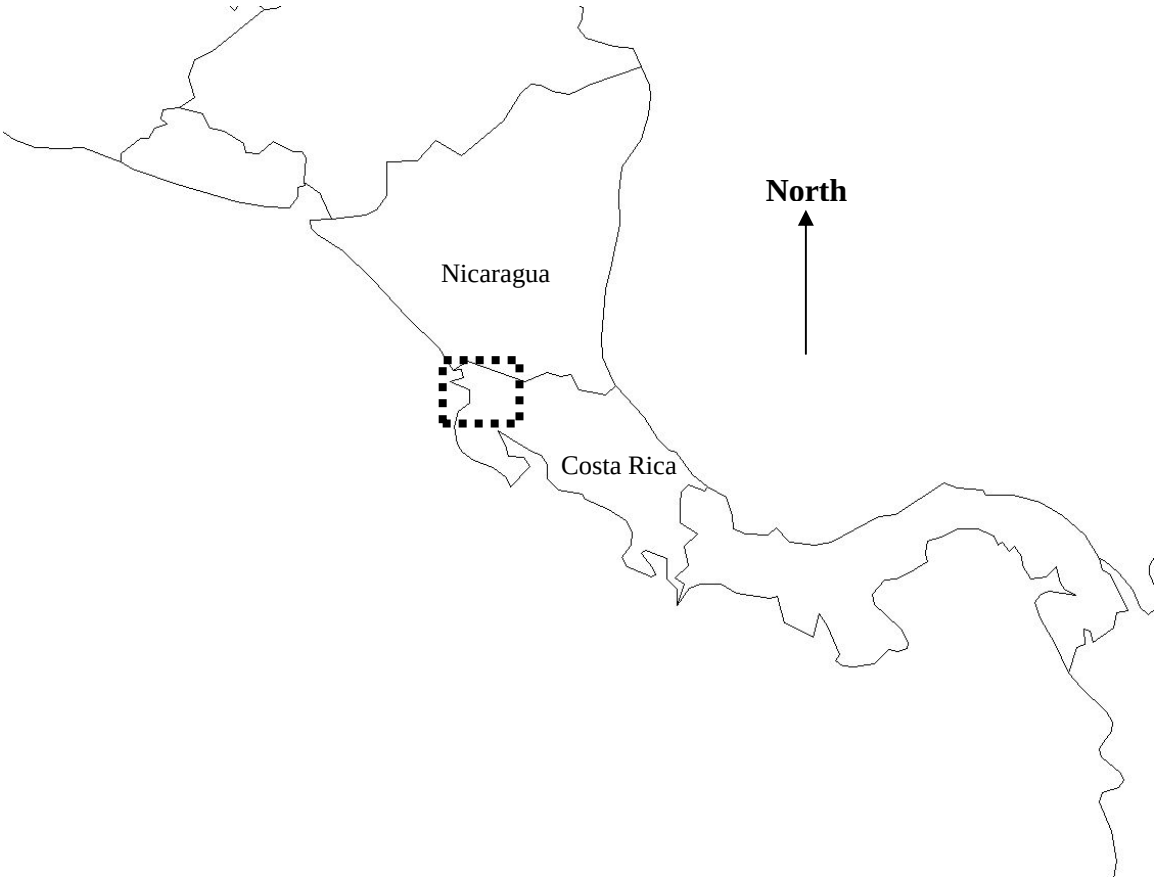


Figure 1.1B.

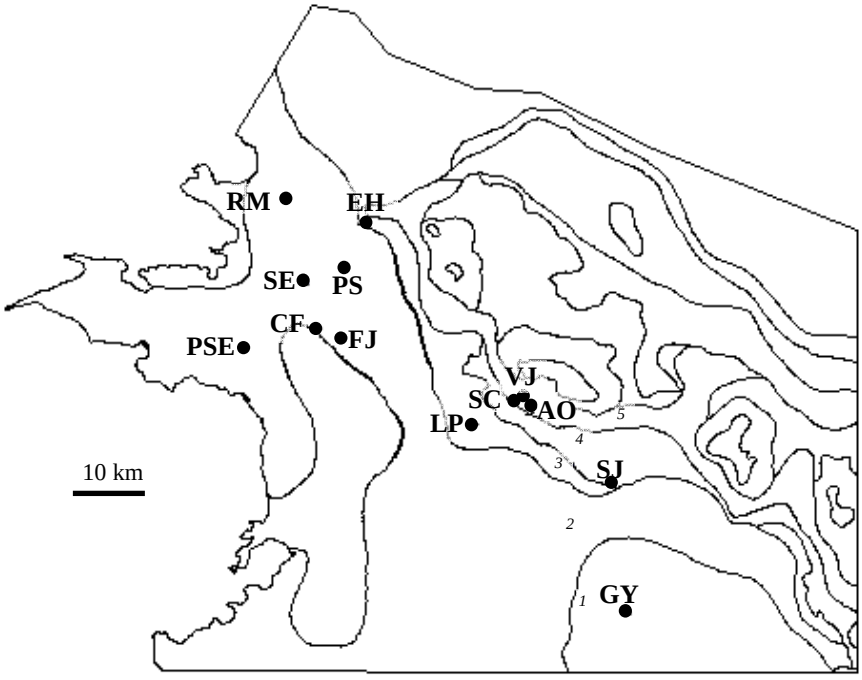


Figure 1.1C.

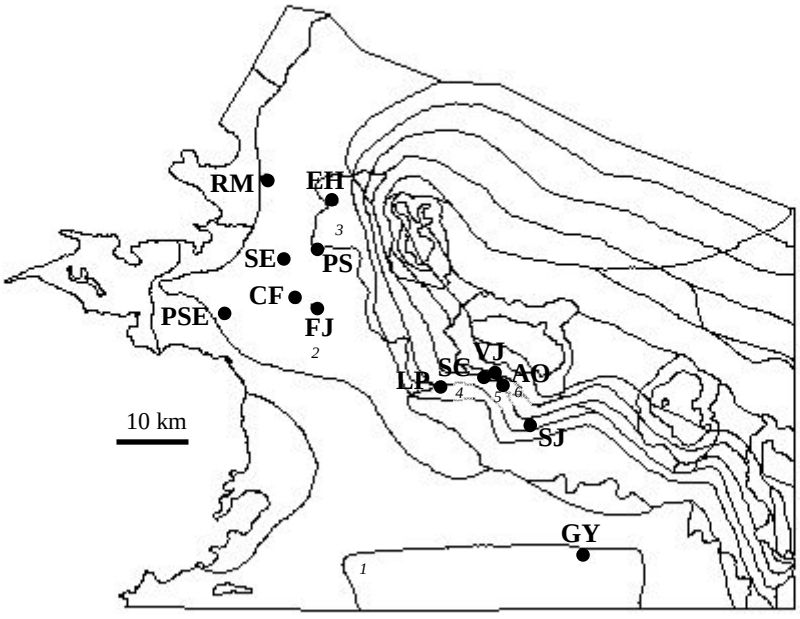


Figure 1.2. Comparison of average number of alleles, average number of private alleles, and average heterozygosity between pre and postfragmentation cohorts.

Figure 1.2.

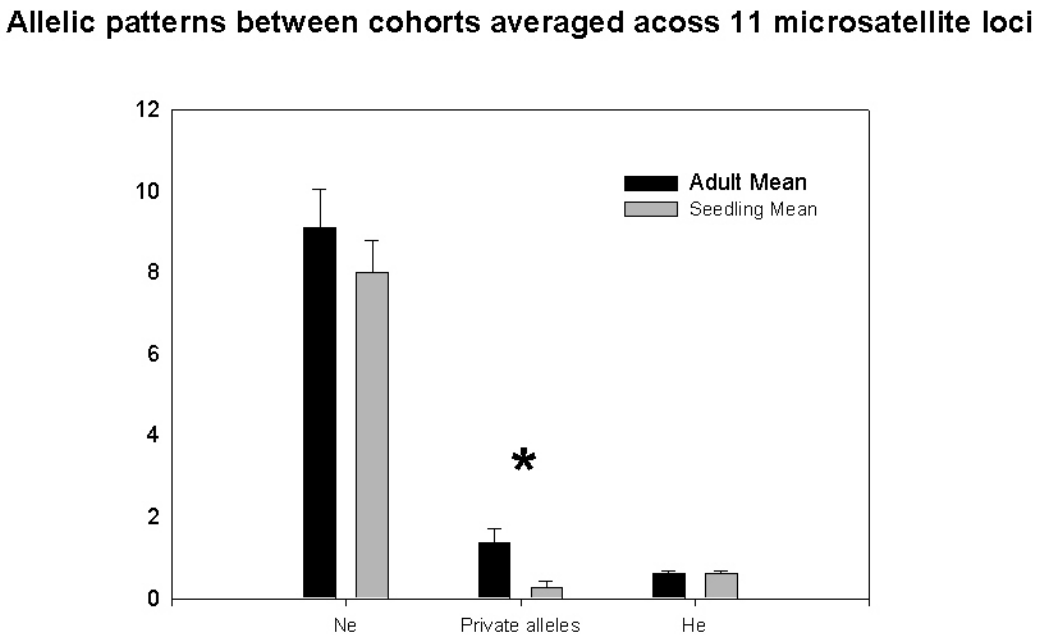

Allelic patterns between cohorts averaged acoss 11 microsatellite loci
12
10
8
6
4
2
0
Adult Mean
Seedling Mean
*
Ne
Private alleles
He

Figure 1.3A. UPGMA tree using Nei's genetic distances among 13 populations. Branches proportional to branch length.

Figure 1.3B. UPGMA tree using Nei's genetic distances among 13 populations of pre-fragmentation cohort. Branches proportional to branch length.

Figure 1.3C. UPGMA tree using Nei's genetic distances among 13 populations of post-fragmentation cohort. Branches proportional to branch length.

Figure 1.3A.

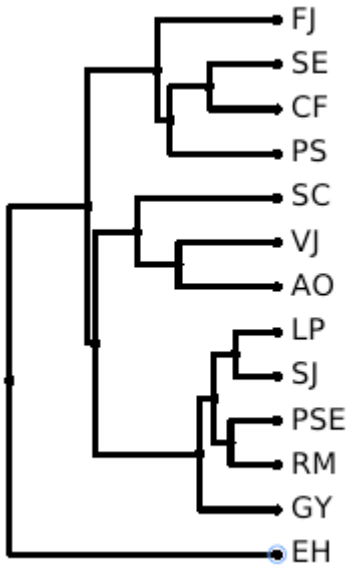


Figure 1.3B.

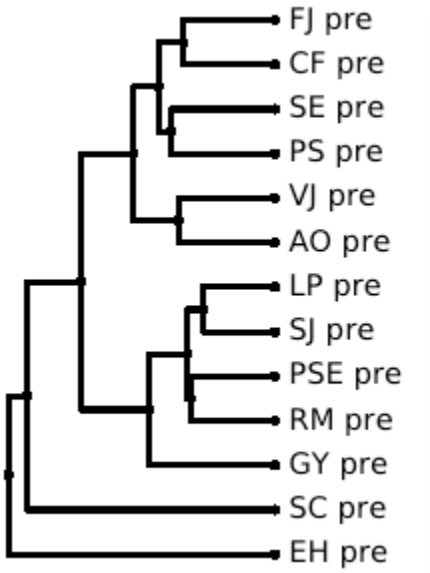


Figure 1.3C.

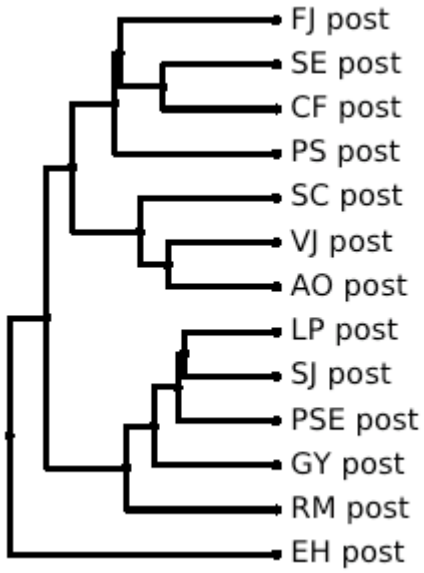


Figure 1.4A. Proportion of individuals from 13 sites assigned to the 5 populations identified by structure (average ln(P|X) = -6970 for K=4, -6875 for K=5, and -6972 for K=6 for three runs at each K).

Figure 1.4B. Same as 5A after excluding ZAG 15 from analysis.

Figure 1.4.

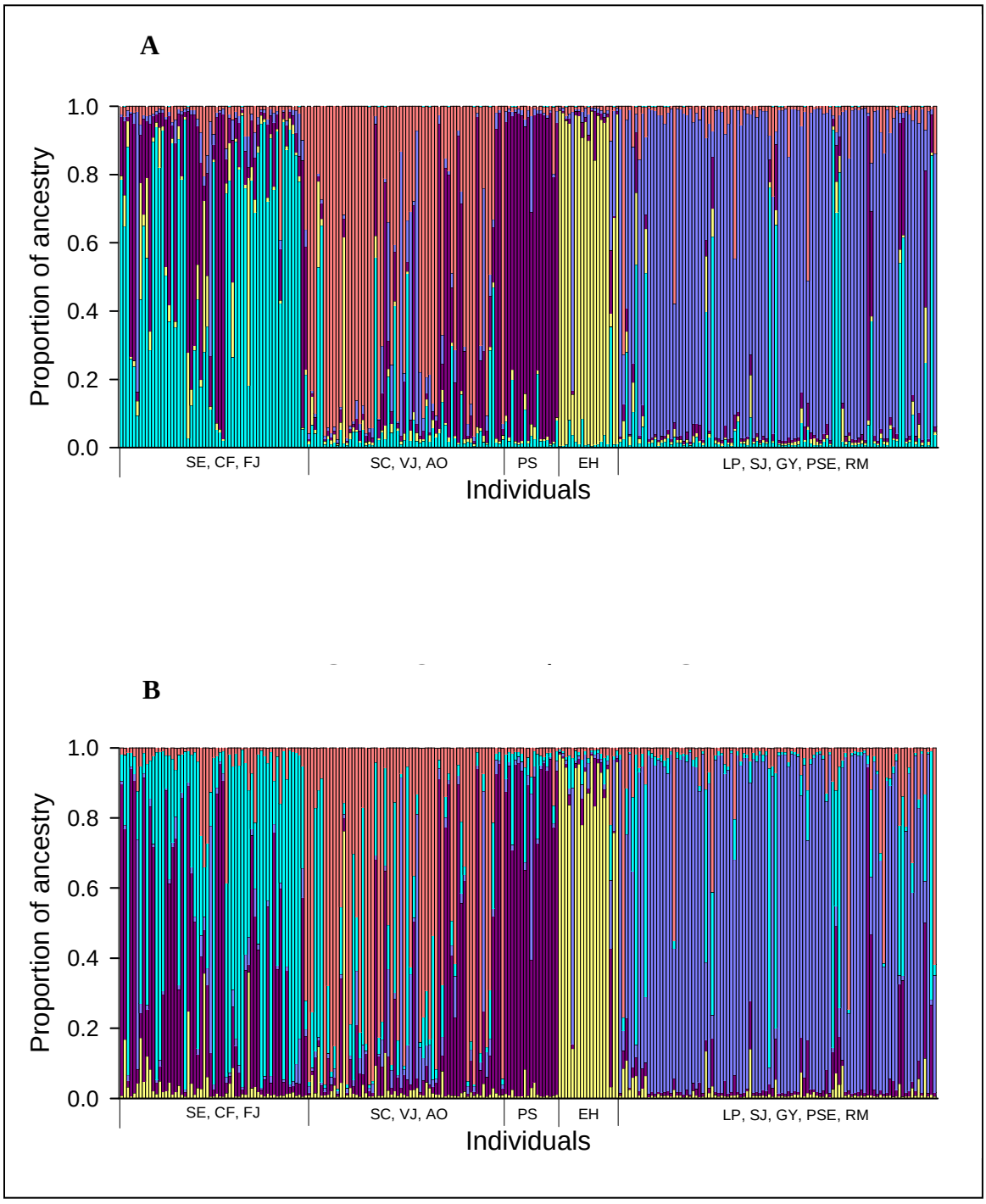

A
Proportion of ancestry
1.0
0.8
0.6
0.4
0.2
0.0
SE, CF, FJ
SC, VJ, AO
PS
EH
LP, SJ, GY, PSE, RM
Individuals
B
Proportion of ancestry
1.0
0.8
0.6
0.4
0.2
0.0
SE, CF, FJ
SC, VJ, AO
PS
EH
LP, SJ, GY, PSE, RM
Individuals

Figure 1.5A) Proportion of prefragmentation individuals from each site assigned to each of five proposed clusters (colors) by STRUCTURE.

Figure 1.5B) Proportion of postfragmentation individuals from each site assigned to each of four proposed clusters (colors) by STRUCTURE.

Figure 1.5.

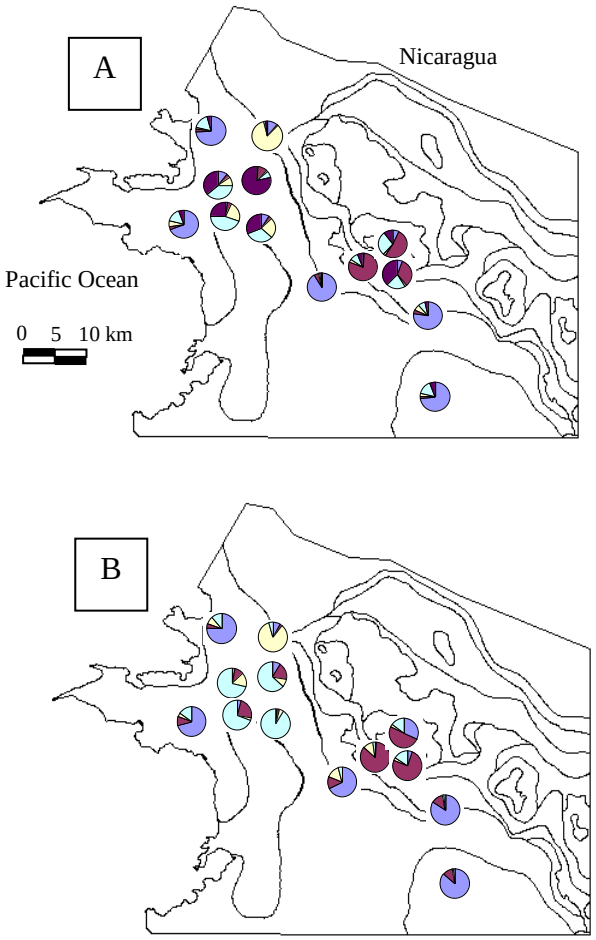


Figure 1.6. Correlogram from spatial autocorrelation analysis. Solid line indicates the autocorrelation coefficient, *r* (-1 to +1), between individuals at 7 km intervals. Dashed lines are 95% confidence intervals based on 1000 random permutations of all individuals. Values above and below dashed lines indicate significant spatial structure. 95% confidence intervals from 1000 permutations are shown for the observed autocorrelation coefficient. The observed r falls significantly above (7, 49, and 70 km) and below (21, 28, 35, 42, 56 km) the dashed line for at leas one of the cohorts, which represents the null hypothesis of no spatial genetic structure, indicating that spatial distance does not consistently predict genetic distance across spatial scales. Therefore, drift alone does not explain population genetic structure of *Q. oleoides* forests. Stars at 14, 35, 49, 56 indicate a significant difference between cohorts.

Figure 1.6.

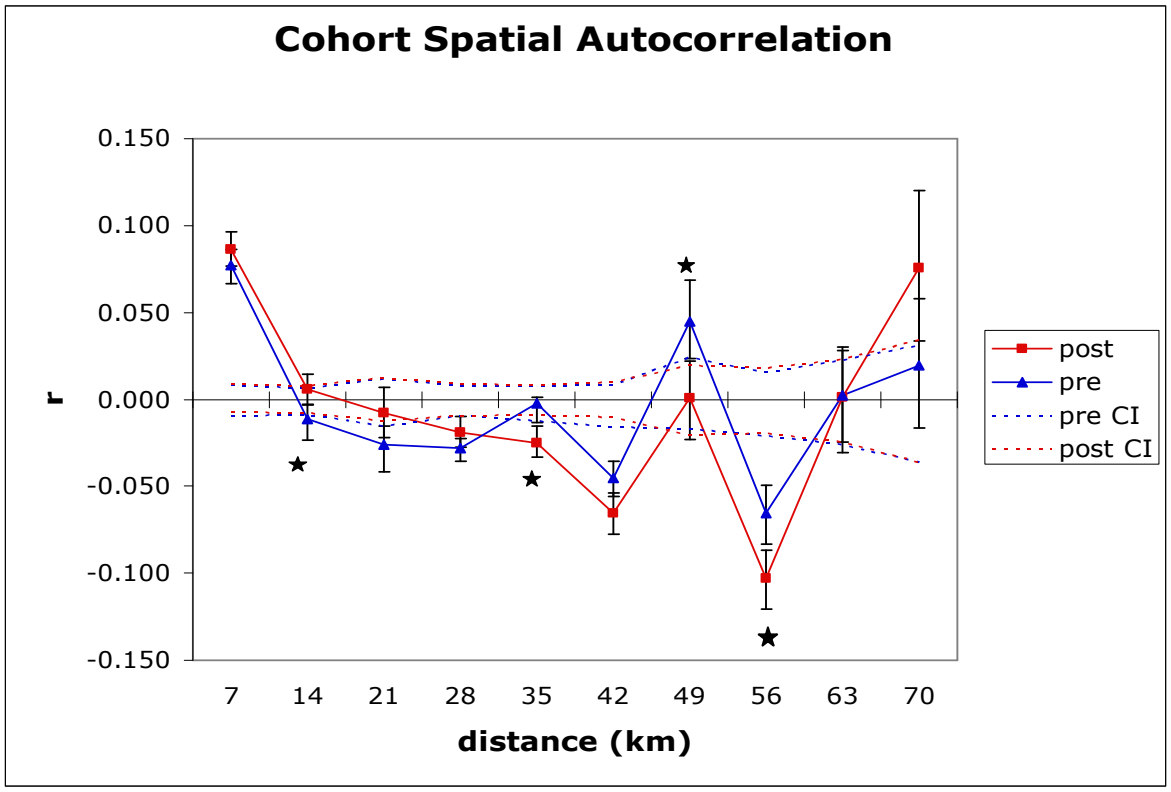
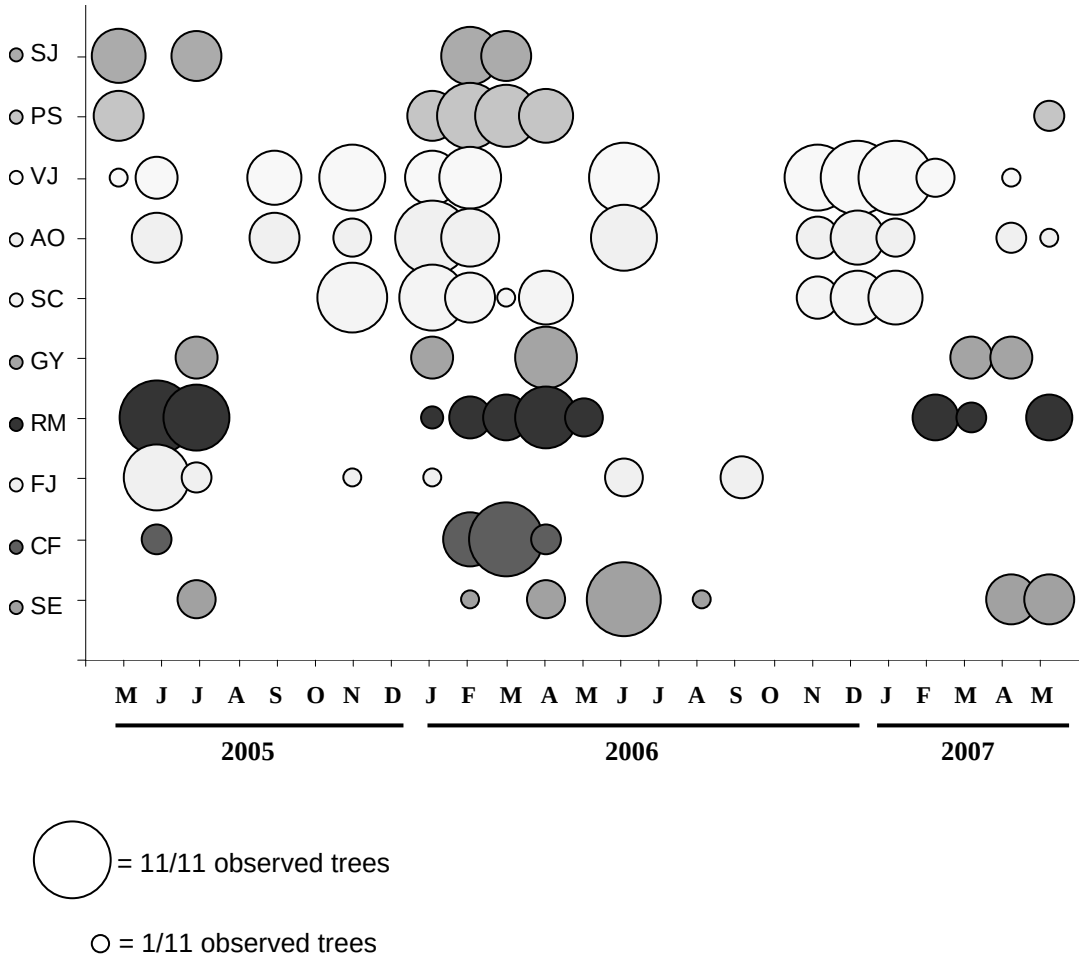

Cohort Spatial Autocorrelation
0.150
0.100
0.050
0.000
-0.050
-0.100
-0.150
r
7
14
21
28
35
42
49
56
63
70
distance (km)
post
pre
pre CI
post CI

Figure 1.7. Presence of male flowers during monthly observations at 10 sites over two years. Size of circle reflects proportion of the 11 monitored trees that were observed with male flowers.

Figure 1.7.


SJ
PS
VJ
AO
SC
GY
RM
FJ
CF
SE
M J J A S O N D J F M A M J J A S O N D J F M A M
2005
2006
2007
= 11/11 observed trees
= 1/11 observed trees

CHAPTER 2

POLLEN DISPERSAL AMONG FRAGMENTED AND GENETICALLY DISTINCT TROPICAL LIVE OAK (*QUERCUS OLEOIDES*) POPULATIONS IN GUANACASTE, COSTA RICA

Wind dispersed pollen has the potential to connect geographically separate populations and prevent divergence at neutral loci via genetic drift. In the seasonally dry tropical oak forests of Guanacaste, Costa Rica, wind pollinated *Quercus oleoides* trees occur in dense stands that became severely fragmented within the past 150 years through the conversion of forest into cattle pastures. In this study, we set out to quantify pollen movement among these populations using a variety of tools. We used a combination of two molecular approaches and pollen trapping to understand how far pollen is dispersed and how the genetic composition of the pollen pool differs throughout the landscape. The TwoGener approach identified significant variation in the pollen pools sampled among mothers (AMOVA, ϕ_{FT} = 0.131 $p<0.001$) and an average of 3.8 effective pollen donors (N_{ep}) per mother tree. Assuming a normally distributed dispersal curve, these results allowed us to estimate an average pollen dispersal distance of approximately 8 meters. A paternity exclusion analysis at one site using the program FaMoz corroborated these short dispersal distance estimates by suggesting that 24 of 29 offspring from a central mother tree came from known fathers within a 50 meter radius. We physically trapped pollen moving throughout that landscape that traveled much greater distances but our interpretation is an extremely high proportion of progeny are fathered by nearby trees. These results are similar to findings for other oaks and other wind pollinated species. Short pollen dispersal distances help to explain how

population genetic structure is maintained in the region. These interpretations are further discussed in the context of our observations of highly variable flowering times among populations and individuals. We include observations on the presence of pistillate flowers in this chapter because the pollen dispersal that we measured was that which was deposited on receptive stigmas and yielded a viable fruit.

Introduction

Gene flow is one of the primary evolutionary processes that shape population genetic structure. Deciphering the movement of gametes and progeny is necessary not only to understand the historical forces that influenced contemporary population genetic structure, but in attempting to predict population viability in the face of environmental changes (Jump *et al.* 2006). In the seasonally dry forests of Guanacaste, Costa Rica, tropical live oak (*Quercus oleoides*) occurs in high-density, remnant populations of once contiguous tropical forest.

These remnant populations contain geographically subdivided genotypes that pre-exist the fragmentation process that has occurred over the past 150 years (Chapter 1). The effects of fragmentation on genetic diversity and structure are subtle, but the future of the *Q. oleoides* forests depends on contemporary gene flow. Seed movement is limited to very short distances due to the poor dispersal behavior displayed by the primary mammal disperser, *Dasyprocta punctata* (Central American Agouti) (Hallwachs 1994). Therefore, the movement of wind dispersed pollen provides critical information for understanding how historical population genetic structure may have shaped, and perhaps how that structure may change due to fragmentation.

The movement of pollen and the likelihood of fertilization depend on the spatial arrangement of individuals across a landscape and the distance that viable pollen travels (Robledo-Arnuncio & Austerlitz 2006). In outcrossing

species, these factors, as well as the degree of flowering synchrony (Nakanishi *et al.* 2005; Smouse *et al.* 2001), contribute to the spatial arrangement of genotypes. The prevailing assumption about how habitat fragmentation affects pollen dispersal is that average dispersal distances decrease because individuals are farther apart. This causes forest fragments to become more isolated, and the potential for extinction through stochastic events or inbreeding depression increases (Bacles *et al.* 2004; Sork & Smouse 2006; Ghazoul 2005; Fuchs *et al.* 2003; Knapp *et al.* 2001).

Several studies have shown this prediction to be satisfied. Where fragmentation has reduced pollen availability, parental fitness and offspring viability have been shown to decline (Wagenius *et al.* 2010; Ashman *et al.* 2004; Nason & Hamrick 1997). An increasing number of studies of fragmented landscapes, however, do not uphold these predictions. For example, pollen dispersal distances have been found to increase after fragmentation in some cases, thereby counteracting the negative effects of increased geographic isolation (Bacles *et al.* 2005; White *et al.* 2002; Fore *et al.* 1992). These studies included animal pollen vectors that were forced to forage greater distances, and wind-dispersed pollen.

Many studies have investigated pollen dispersal distance in the genus *Quercus* (Dow & Ashley 1998; Dow & Ashley 1996; Fernandez-Manjarres *et al.* 2006; Fernandez & Sork 2005; Streiff *et al.* 1999; Sork *et al.* 2005). In many of these studies, nuclear genetic markers were employed to infer the paternal

contribution to offspring genotype (Smouse & Sork 2004). This type of "direct measurement" differs from earlier approaches at quantifying gene flow. Earlier "indirect estimates" of pollen dispersal distances were inferred from the distribution of alleles throughout a population using Wright's F-statistics. Indirect estimates, however, rely on assumptions of equilibrium between gene flow, mutation, and drift which are unlikely to be true in most field settings, especially in recently disturbed landscapes (Whitlock & McCauley 1999). More accurate estimates of contemporary pollen movement are made using the direct molecular techniques that determine the paternal and maternal genetic contribution to each individual offspring. Dispersal distance is then estimated by measuring interparental distances or by providing a distance where some known proportion of parents are found (Smouse & Sork 2004).

Paternity analysis, and parentage-type methods in general, attempt to match a specific father to each offspring. This task quickly becomes overwhelming with increasing numbers of potential pollen donors, so likelihood methods were developed to calculate the probability that certain individuals can be excluded as likely fathers (Gerber *et al.* 2003). In this paternity exclusion approach, a large number of offspring and all potential fathers are genotyped within a defined radius of a central mother tree (Adams *et al.* 1992). Even this approach remains somewhat unsatisfying, however, because the distance that any pollen traveled from outside the prescribed radius remains unknown (Smouse & Sork 2004). Alternatively, a method called TwoGener uses only

maternal and offspring genotypes to infer the paternal contribution. The within and among mother variation in the inferred paternal gametes is compared and the pollen dispersal distance is equal to the distance between mothers that are sampling distinct pollen pools (Smouse *et al.* 2001).

The primary objective of this study was to quantify pollen dispersal distance using both direct and indirect molecular methods, as well as physical pollen trapping, in order to understand better the role of gene flow in shaping the current population genetic structure of *Q. oleoides* in Costa Rica. Historical patterns of pollen movement may have been altered by severe fragmentation during the last century. Current gene flow may foreshadow changes to the frequency and distribution of genotypes throughout the landscape. We used nuclear microsatellite markers to: 1) quantify the amount of among mother variation in the pollen pool sampled by 10 mother trees throughout Guanacaste, 2) estimate the average number of effective pollen donors per mother tree, and 3) estimate the average pollen dispersal distance using two different methods. To supplement the TwoGener results, we conducted a paternity exclusion analysis at one site. Lastly, we trapped pollen moving into and out of an isolated stand in order to gauge the potential for long distance pollen movement. This combined approach provides a more comprehensive understanding of pollen movement throughout *Q. oleoides* forests. We discuss these results in the context of the results of our flowering phenology monitoring described in Chapter 1.

Methods

Study site and species

This study was carried out in the Área de Conservación Guanacaste (ACG) of northwestern Costa Rica. Prior to becoming a protected area, the region experienced significant anthropogenic forest conversion to cattle pastures (Janzen 1983). Tree species in tropical forests are primarily animal pollinated and are usually rare, but *Quercus oleoides* is wind pollinated and occurs in high density stands. Its distribution extends from the Gulf Coast of Mexico to the Pacific Coast of Northwest Costa Rica. The Costa Rican live oaks are genetically and ecologically distinct from the rest of the range (Cavender-Bares *et al.* in review) and we have found significant spatially segregated genetic structure at this scale (Chapter 1, Figure 2.1A).

Pollen dispersal distance estimation

Pollen dispersal distances were estimated through molecular characterization of individuals using nuclear microsatellite markers. Methods for DNA extraction, amplification, and genotyping followed Chapter 1. In this study we assayed eight microsatellite loci developed for other species in the genus *Quercus* by Kampfer (ZAG 30 in *Q. robur*) and Steinkeller (ZAG 110, ZAG 15, ZAG 9, ZAG ½, ZAG1/5, ZAG102, ZAG 35 in *Q. petraea*) (Steinkellner *et al.* 1997; Kampfer *et al.* 1998). We employed two computational methods for

estimating pollen dispersal distance based on microsatellites: 1) the TwoGener (two generation) method of Smouse and others (Smouse *et al.* 2001; Austerlitz & Smouse 2001a; Austerlitz & Smouse 2001b; Austerlitz & Smouse 2002; Dyer *et al.* 2004) and 2) the paternity exclusion methods found in the program FaMoz by Gerber et al. (2003). Finally, we trapped pollen around an isolated forest fragment to measure dispersal directly. The TwoGener method has limitations in that ϕ_{FT} can be upwardly biased by relatedness among individuals and by highly structured genetic variation across a landscape. A benefit of the TwoGener approach, however, is that it requires genotyping fewer offspring than parentage methods. The mother trees are effectively acting as pollen traps and the heterogeneity of their sampled pollen pools is compared. Parentage methods are advantageous in that distances of fathers can be accurately established with thorough sampling. A disadvantage is that they often yield only minimal distances because much pollen arrives from outside the specified radius. By employing both the TwoGener and parentage methods, a more informative picture of the shape of the pollen dispersal decay curve can be drawn and long distance gene flow can be better inferred (Smouse & Sork 2004).

TwoGener analysis

The authors of the TwoGener method suggest sampling approximately 20 offspring from each mother for a balance between collection effort, resources, and information. We genotyped leaf tissue from 22 germinated acorns from each

of ten variably spaced mother trees (Figure 2.1B and 2.1C). Maternal and progeny genotypes were characterized by assaying the eight microsatellite markers described above. The mother trees were chosen from throughout the landscape so that multiple distance comparisons can be made. A genetic distance matrix was constructed to estimate intergametic genetic distances of pollen, and an analysis of molecular variance (AMOVA) was used to determine variation in pollen donors within one tree and between pairs of trees. The fraction of total variance accounted for by interfemale distance is estimated by ϕ_{FT} (which is analogous to F_{ST} for male gametes) using the spatial heterogeneity in male gametes. ϕ_{FT} is inversely proportional to mean pollination distance and provides an estimate of effective pollination neighborhood size (Smouse *et al.* 2001; Dyer *et al.* 2004). The TwoGener procedure was implemented in GenAlEx (Peakall & Smouse 2006). TwoGener also provides a number of effective pollen donors (N_{ep}) by breaking the ϕ_{FT} measurement down as follows (Austerlitz & Smouse 2001a):

$$\Phi_{FT} = \frac{Q_0 - Q(\bar{z})}{2 - Q(\bar{z})}$$

where Q_0 is the probability that two pollen grains that fertilized ovules in the one mother come from the same father, and $Q(\bar{z})$ is the probability that two pollen grains drawn from two different mothers come from the same father. Q_0 is

equivalent to $1/N_{ep}$ (N_{ep} is the effective number of pollen donors). TwoGener also uses the Austerlitz and Smouse (2001) approach to estimate dispersal distance (d) from the following equation for N_{ep}: $N_{ep} = 8d^2\Delta$ (for a bivariate normal distribution) and Δ = stem density per hectare. There is considerable debate about the proper distribution to use to model pollen dispersal (Austerlitz *et al.* 2004). Questions remain about the fatness of the tail for the actual distribution. We took a conservative approach, where the tail is skinny, in using the bivariate normal distribution here as recommended by TwoGener's authors.

Paternity exclusion

At one site, 63 adult trees (over 20cm DBH) within a 50 meter radius of a central mother tree were genotyped at eight microsatellite loci (Figure 2.1B). Leaf tissue from the mother tree and 29 progeny were also genotyped. We used the program FaMoz to calculate exclusion probabilities using likelihood methods to estimate the number of likely pollen donors from within the 50m radius (Gerber *et al.* 2003). The method uses the microsatellite allele frequencies to compare the likelihood ratio between all possible father-offspring pairs and the observed data. The higher the likelihood score for any particular parent-offspring pair, the more likely it is true. A threshold likelihood score was developed through simulating progeny by random association of gametes, and paternity was attributed to the pollen pool coming from outside the stand in observed likelihood scores fell below the threshold value.

Pollen trapping

In order to better understand how far pollen was capable of moving (not just how far it was moving on average to produce offspring), we devised a novel method where we cut a 4 cm diameter hole from a notecard and placed clear packaging tape over the hole on one side. This "pollen trap" was then attached to a 50 cm tall post and the post was placed in the ground at various distances and orientations around an isolated forest fragment (Figure 2.2A). The remnant forest patch consisted of approximately 15 adult *Q. oleoides* trees and was surrounded by pasture grass on all sides with no other and *Q. oleoides* trees within approximately 1 km. Pollen traps were placed on the northwest, northeast, southwest, and southeast side of the remnant forest patch to take maximize exposure to prevailing wind during the second week of July, 2007 when most of the trees in the region were flowering. On the four sides traps were placed at 10m, 20m, and 50m from the forest patch with the sticky side facing into the remnant to collect pollen blowing out for three days. Four pollen traps were also placed at the edge of forest fragment on the same four sides facing out into the pasture in order to catch pollen blowing in over three days. After three days the tape was placed on glass microscope slides, and the *Q. oleoides* pollen grains were counted with the aid of a reference collection of *Q. oleoides* pollen grains used for confirming species identity. Orientation and sidedness may not

accurately indicate the direction of pollen movement due to eddying currents and vortices.

Phenology

The presence of male and female flowers was monitored at 10 sites as described in Chapter 1. In this chapter, we include a discussion of these data in the context of the pollen movement analyses. Because of the small size of the pistillate flowers, the certainty of their absence is in question. Our observations were primarily on lower limbs of open grown trees, but in some cases the presence of canopy pistillate flowers is unknown.

Results

Genotyping of the individuals in both the TwoGener analysis and the paternity exclusion analysis yielded high diversity and 100% polymorphic loci. The number of alleles per locus ranged from 6 (ZAG ½) to 20 (ZAG 102). This provides sufficient variation to differentiate potential fathers and offspring.

TwoGener analysis

We were able to successfully genotype a total of 200 offspring from 10 mothers. The inter-mother distances varied from less than one kilometer to over 60 km (Figure 2.1C). The majority of the total variation in male gametes (87%) was found within mothers, but a statistically significant 13% of the total variation

in male gametes occurred among mothers (ϕ_{FT} = 0.131 p<0.001, Table 2.1). Pairwise ϕ_{FT} values between mothers ranged from 0.04 – 0.23 (Table 2.2). On average only 3.8 pollen donors fathered the 22 offspring per mother. We used the default dispersal curve in TwoGener (bivariate normal) and our own density measurement (81 trees per hectare) to obtain an estimate of eight meters for the average effective pollen dispersal distance.

Paternity Exclusion

The number and diversity of the eight microsatellite markers assayed in these individuals were sufficient to exclude potential fathers with high probability (cumulative exclusion probability over 8 loci = 0.997). There was very little chance that any two individuals drawn at random from the population could not be differentiated (Probability of identity <0.001). The likelihood approach of Famoz successfully excluded all but one potential father for 24 of the 29 genotyped offspring. Fathers for five of the 29 offspring were likely found outside of the 50 meter radius of the study stand yielding an inflow rate of approximately 17%.

Pollen trapping

Pollen was successfully intercepted exiting all four sides of the isolated forest fragment over three days. The trap on the southwest side of the stand contained two orders of magnitude more pollen than any other trap nearest to the

stand (~1000 total pollen grains at the nearest trap versus ~40 on the others). The number of trapped pollen grains decreased with increasing distance from the fragment, but even at 50 meters away, the counts were approximately 10 grains (Figure 2.2B). Pollen entering the stand from elsewhere was also captured on all sides, and the northwest trap contained the most (Figure 2.2C). The number of pollen grains coming into the stand, however, was roughly similar to the number intercepted at 50 meters coming out of the stand. The results indicate prevailing wind direction from the northerly sides of the fragment and pollen entering the stand from trees at least 1 km away.

Phenology

The results of the monitoring of the less conspicuous pistillate flowers indicate that they are much more seasonal in their appearance than the staminate flowers shown in Chapter 1 (Figure 2.3A). By their nature, these organs are more difficult to observe from ground observations so the accuracy of these data is unknown. We did, however, observe females flowers in all sites in at least one month over the 24 months of observations (Figure 2.3B).

Discussion

The results of our two molecular analyses provide evidence for very short distance pollen movement in *Quercus oleoides*. The TwoGener analysis showed an average effective dispersal distance of eight meters and the paternity analysis

indicated that pollen donors for 83% of progeny likely come from within a 50 meter radius. Other studies that utilized both of these types of analyses have observed a discrepancy between the where the paternity method sometimes yields greater estimated distances (Smouse & Sork 2004), but our results seem to support one another.

The low number of effective pollen donors per mother, 3.8, is comparable to other *Quercus* species in disturbed landscapes (e.g. Nep = 3.68 for *Q. lobata*, (Sork *et al.* 2002), Nep = 5.1-6.1 for *Q. humboldtii* (Fernandez & Sork 2005), and Nep = 8.22 *Q. alba* (Smouse *et al.* 2001). Our estimated average pollen dispersal distance of 8m for *Q. oleoides* is on the low end in comparison with other Quercus species (17m for *Q. alba* and 65m for *Q. lobata*). The large difference in estimated pollen dispersal distance between *Q. lobata* and *Q oleoides* (65m and 8m) can be explained by the higher density of *Q. oleoides* (1.19/hectare and 81/hectare). Other wind dispersed species have been shown to similarly derive paternity from nearby individuals (*Fraxinus* (Bacles & Ennos 2008) and *Populus* (Slavov *et al.* 2009)). In all these studies, the possibility for rare long range pollination is still present and much debate continues over appropriate shape for modeling the pollen dispersal curve (Austerlitz *et al.* 2004). The 17% inflow rate, or the proportion of genotyped progeny fathered by individuals outside the sampled radius, observed in the paternity exclusion study is less than those observed in other *Quercus* species as well (e.g. *Q. macrocarpa* = 0.57 (Dow & Ashley 1998).

Year to year variability in pollen production and variability in the timing of flowering may also affect population genetic structure. In a separate study we monitored flowering time and found little synchrony in staminate flowering among sites over two years, but the overlap in presence of flowers among populations was not correlated with genetic distance (Chapter 1). This indicates that these populations are not genetically isolated due to diverging flowering times. The timing of flower production seems to be independent of the genetic variation observed at these neutral loci. The lack of strong seasonality in flowering that we observed indicates that a multiyear study of effective pollination would better elucidate long term patterns of pollen movement, especially given the lifespan and number of reproductive years of this long lived species (Nakanishi *et al.* 2005). Additionally, the frequency of individuals flowering at any one time or in one particular year has an influence on whether or not phenological assortative mating could result (Weis *et al.* 2005). In this chapter we include data on pistillate flowering to illustrate the fact that it is much more seasonal than the pattern of staminate flowering (Figure 2.3A & 2.3B). This indicates that the potential for rare long distance fertilization exists, and a more precise account of the presence of pistillate flowers may yield the expected correlation with genetic similarity among populations.

The effect that forest fragmentation has on pollen dispersal in the region cannot be assessed directly without a comparable non-fragmented area, but the pollen trapping experiments show that pollen is moving much farther than the

average *effective* pollination distance. This provides support for the likelihood of the occasional long distance pollination event and leads to questions of the duration of pollen viability and perhaps recognition of nearby or related pollen by stigmatic surfaces. We have previously shown a high degree of genetic differentiation among these *Q. oleoides* populations, and these results provide a mechanism to explain how that structure could be maintained (Chapter 1). Chapter 1 also showed, however, that post-fragmentation pollen dispersal might be connecting previously isolated populations. We predict this to be most likely in areas where the density of trees has been reduced; fragmentation has probably not affected the pollen pool sampled by mothers in contiguous stands.

Extremely limited pollen dispersal may prevent locally adapted genotypes from being overwhelmed by immigrant pollen and eroding the advantage they have in their specific environment. At this time, however, we do not have evidence to support a local adaptation hypothesis (Chapter 3). The molecular evidence from this study provides support for the predominance of short distance pollen movement, but the pollen trapping does indicate the potential for long range dispersal over the fragmentation matrix. As the methods for measuring the frequency and relative importance of long distance pollen dispersal continue to be refined and improved, so to will our understanding of the reproductive history and future of wind pollinated trees in disturbed habitats. Phenomena such as masting that have been observed to affect flower and seed production in other oak species (Koenig & Knops 2005) has not been reported here but future

studies should continue monitoring of flower phenology with an emphasis on the timing pistillate flower production and receptivity in addition to staminate flowers.

Table 2.1. AMOVA table from Twogener analysis showing a statistically significant 13% of the total variation in paternal gametes occur among mothers.

Source	df	SS	MS	Est. Var.	%
Among Mothers	9	74.356	8.262	0.305	13%
Within Mothers	195	395.369	2.028	2.028	87%
Total	204	469.726		2.332	100%

Table 2.2 *Pairwise population* Φ_{FT} *values*

	30	**45**	**26**	**1**	**3**	**4**	**27**	**54**	**21**	**11**
30	0.000									
45	0.116	0.000								
26	0.087	0.102	0.000							
1	0.130	0.091	0.138	0.000						
3	0.221	0.090	0.125	0.142	0.000					
4	0.230	0.164	0.221	0.189	0.228	0.000				
27	0.196	0.154	0.128	0.147	0.177	0.202	0.000			
54	0.066	0.090	0.088	0.092	0.183	0.143	0.104	0.000		
21	0.112	0.072	0.128	0.101	0.119	0.143	0.075	0.082	0.000	
11	0.068	0.079	0.087	0.071	0.157	0.162	0.121	0.046	0.079	0.000

Mothers are identified by numbers (30, 45, 26, 1, 3, 4, 27, 54, 21, 11) as in Fig 2.1

Figure 2.1A. Previously sampled sites for population genetic structure in *Quercus oleoides*. Colors represent proportional assignment of the 20 individuals at each site assigned to each of five likely genetically distinct clusters based on 11 microsatellite loci.

Figure 2.1B. Sites where mothers and progeny were collected for TwoGener analysis (stars), paternity analysis (circle), and flowering phenology monitoring (squares).

Figure 2.1C. Plot of mothers using UTM coordinates illustrating the distance (meters) between mothers.

Fig. 2.1

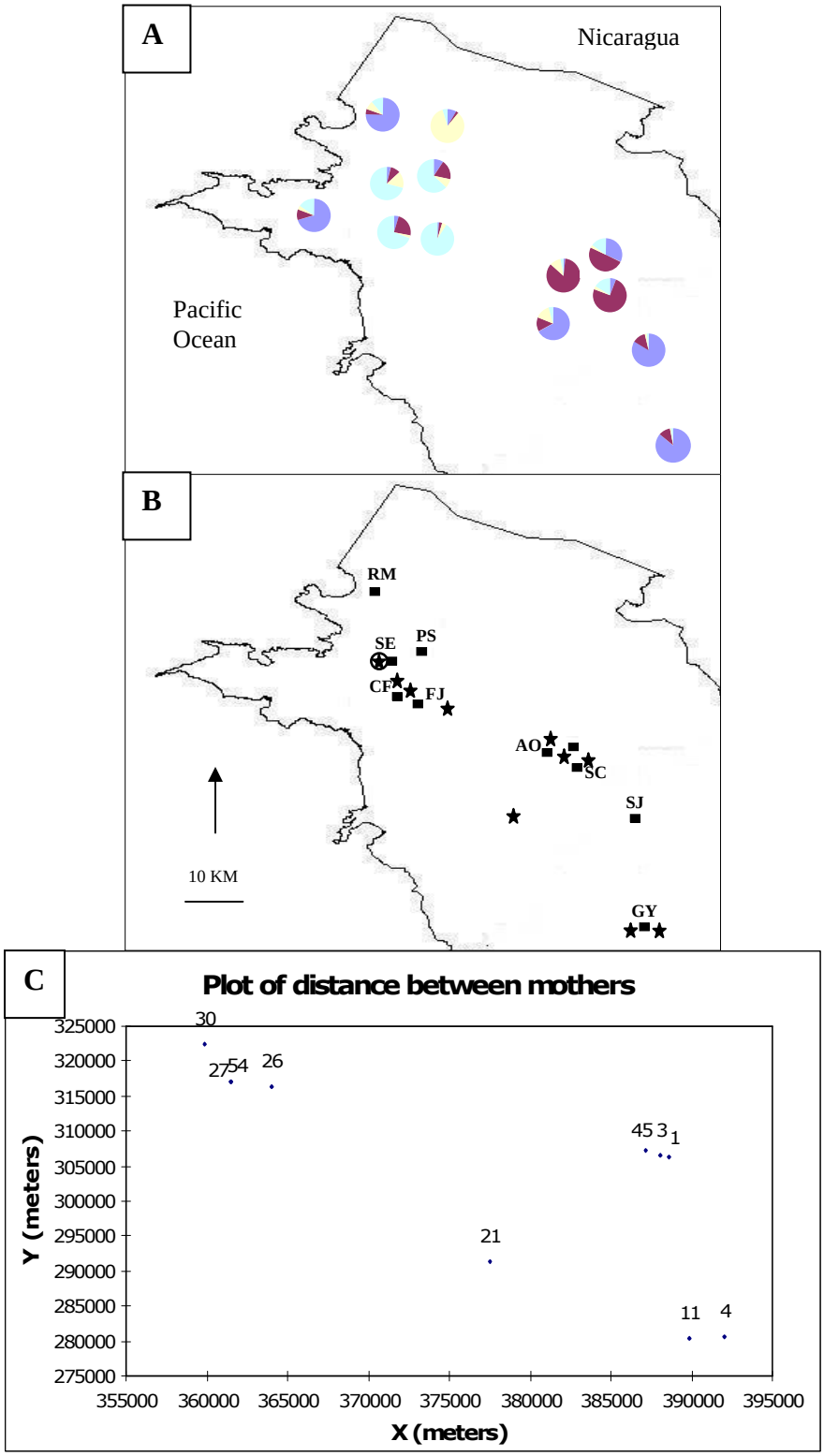
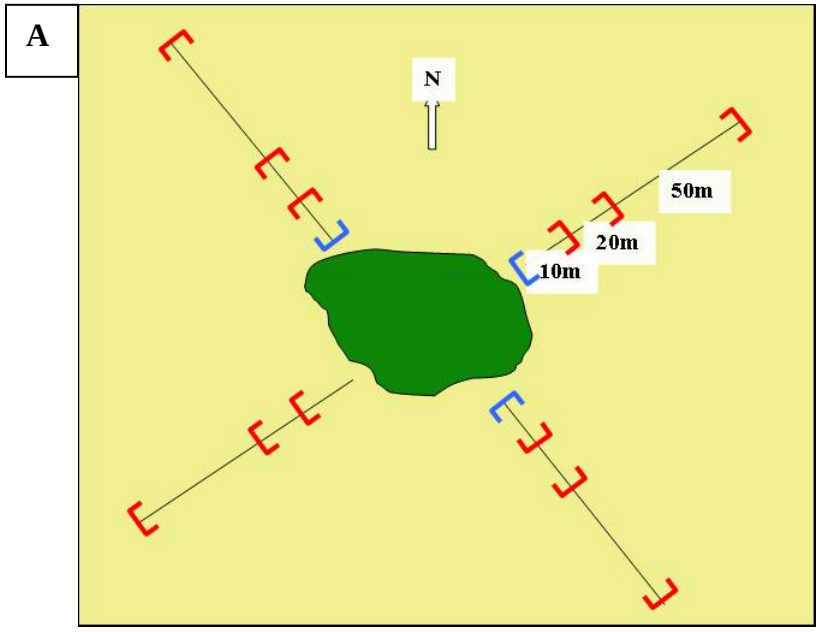

A
Nicaragua
Pacific
Ocean
B
RM
SE
PS
CF
FJ
AO
SC
SJ
GY
10 KM
C
Plot of distance between mothers
Y (meters)
X (meters)
325000
320000
315000
310000
305000
300000
295000
290000
285000
280000
275000
355000
360000
365000
370000
375000
380000
385000
390000
395000
30
27
54
26
45
3
1
21
11
4

Figure 2.2A. Illustration of pollen traps facing into the forest fragment (red) and away (blue).

Figure 2.2B. Number of *Quercus oleoides* pollen grains counted on pollen traps facing into the forest fragment at various distances out into the pasture in four directions.

Figure 2.2C. Number of *Quercus oleoides* pollen grains counted on traps facing away from the forest fragment in four directions.

Fig. 2.2


A
N
50m
20m
10m

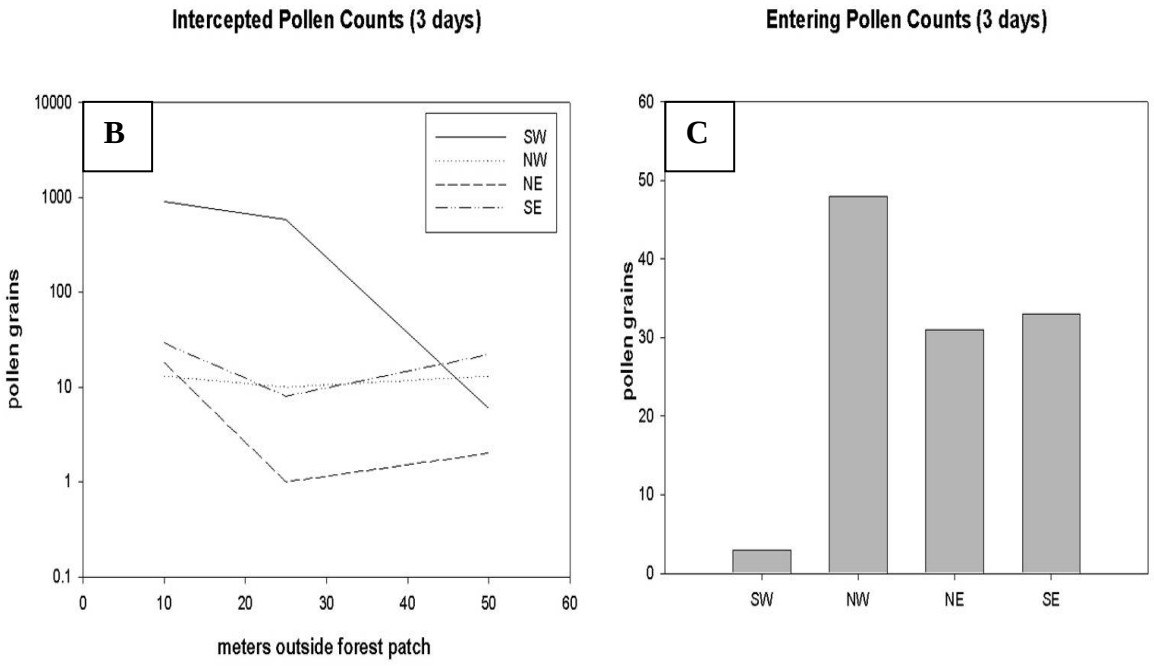
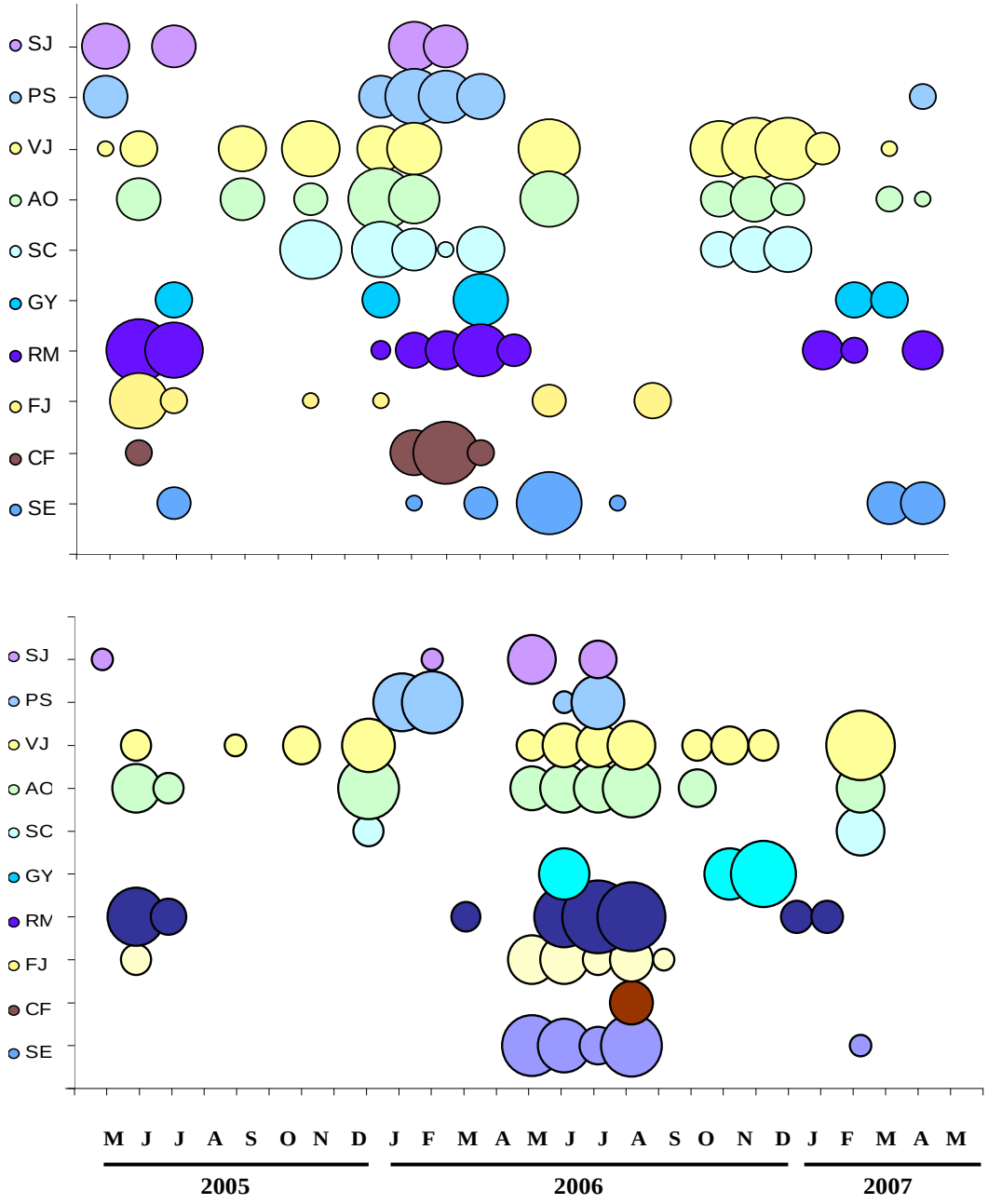

Intercepted Pollen Counts (3 days)
Entering Pollen Counts (3 days)
B
SW
NW
NE
SE
pollen grains
10000
1000
100
10
1
0.1
0
10
20
30
40
50
60
meters outside forest patch
C
pollen grains
60
50
40
30
20
10
0
SW
NW
NE
SE

Figure 2.3A. Months where staminate flowers were observed on *Quercus oleoides* trees at 10 sites.

Figure 2.3B. Months where pistillate flowers were observed on Quercus oleoides trees at 10 sites. Size of circle is represents to the proportion of observed trees where male flowers were present.

Fig. 2.3


SJ
PS
VJ
AO
SC
GY
RM
FJ
CF
SE
SJ
PS
VJ
AC
SC
GY
RM
FJ
CF
SE
M J J A S O N D J F M A M J J A S O N D J F M A M
2005
2006
2007
= 11/11 observed trees flowering
= 1/11 observed trees flowering

CHAPTER 3:

INVESTIGATING LOCAL ADAPTATION IN GENETICALLY DISTINCT *QUERCUS OLEOIDES* SEEDLING POPULATIONS IN CONTRASTING ENVIRONMENTS

We established a reciprocal transplant experiment between maternal families of seedlings from high and low elevation populations of tropical live oak (*Quercus oleoides*) in order to test for local adaptation. Lowland areas of Guanacaste Province, Costa Rica experience a four to five month dry season that is much more severe than upland areas. Likelihood ratio tests comparing Aster models of increasing complexity showed that the effects of family and garden were highly significant ($p<0.001$), but there was no interaction between family and garden ($p=0.15$). The model that included a population x garden interaction did not fit the data significantly better than a model without that interaction, indicating no evidence for local adaptation. Fitness was much higher for both populations in the upland garden where dry season precipitation amounts are greater. The upland population, however, had a lower fitness estimate than the lowland population in both gardens. These results suggest that drought during the dry season imposed strong selection on seedlings in the lowland environment; but plasticity in lowland populations enables those individuals to take advantage of the less severe environment in the upland. The magnitude of the fitness difference decreased from 2007 to 2008 to 2009. In our comparison of acorn size from a subset of upland and lowland families did not indicate a significant difference, however, so any maternal effect that might be involved does not appear to be related to acorn size. We interpret these results to indicate that the infrequent long distance pollen dispersal that we reported in

Chapter 2 has occurred between these upland and lowland populations and prevented the native population from gaining a selective advantage.

Introduction

The concept of adaptive differentiation among populations, or local adaptation by natural selection, receives enormous attention in the ecological and evolutionary literature because of its usefulness in explaining phenomena such as speciation, diversification, and range limits (reviewed in Hereford 2009; Kawecki & Ebert 2004). The reciprocal transplant experiments that are commonly employed to answer these types of questions are also informative in predicting responses to climate change (Etterson 2004a; Etterson 2004b). The particular result that provides the evidence for local adaptation in nearly all these studies is a higher fitness measurement for native individuals or populations than immigrants, as evidenced by a significant population by habitat interaction in an analysis of variance (Linhart & Grant 1996; Kawecki & Ebert 2004).

The following three conditions are among a number of circumstances where one could expect to find evidence for local adaption: 1) A high amount of genetic variation is present in the species or population; 2) Populations occur over varying environmental conditions; 3) Gene flow is extremely limited, absent among areas of the range, or just less than the impact from selection (Reznick & Ghalambor 2001; Holt & Gomulkiewicz 1997). Genetic variation provides the raw material that allows species to take advantage of novel environments and this variation is maintained among populations by variable environmental conditions (Manel *et al.* 2003), (Ellstrand & Elam 1993). Gene flow among populations can be interrupted by a plastic change in the timing of flower production caused by

variability in the necessary abiotic environmental cues (Hall & Willis 2006; Tarayre *et al.* 2007; Schemske & Bierzychudek 2007), or a genetic change that occurs when a population invades a new habitat (Levin 2009).

Species with ranges that span large geographic and environmental distances are common subjects of local adaptation experiments (Lowry *et al.* 2008; Joshi *et al.* 2001). Reciprocal transplant experiments are often performed on short-lived perennial or annual plants because their life history allows for the lifetime fecundity of each individual to be measured (Etterson 2004a). This is not a required condition, however, because when individual fecundity cannot be measured, such as in long-lived perennials and trees, traits relating to size or growth have been shown to be effective fitness surrogates (Ramirez-Valiente *et al.* 2009; Shaw *et al.* 2008; Saenz-Romero *et al.* 2006).

In Chapter 1 we reported on a widespread long-lived perennial plant with a significant amount of genetic differentiation among neutral markers occuring over a steep environmental gradient. *Quercus oleoides* is a common canopy tree in much of the dry topics (Boucher 1983). At its most extreme high and low range in the Guanacaste Province of northwestern Costa Rica, abiotic environmental conditions are decidedly disparate (Figure 3.1A and 3.1B). The most obvious difference is that lowland areas undergo a much more severe dry season than upland populations. We found high amounts of differentiation at neutral markers between these environments (Chapter 1), and we have also found average effective pollen dispersal distances to be quite low (Chapter 2). Therefore, this

system presents conditions that could allow for adaptive differentiation to develop. In this study we tested for local adaptation by establishing an upland and lowland garden of reciprocally transplanted maternal families of *Q. oleoides* seedlings. The genetic differentiation among populations, the steep environmental gradient, and the limited pollen dispersal distances lead us to predict that populations have adapted to their native environment and, therefore, have higher fitness than immigrants.

Methods

Seedling and garden establishment

We collected at least 50 *Quercus oleoides* acorns from 47 trees representing three populations around Guanacaste Province, Costa Rica. Thirteen maternal families were collected from lowland areas near sector Santa Rosa of the Área de Conservación Guanacaste (ACG) and thirteen maternal families were collected from upland areas near sector Pailas (Figure 3.2). Additionally, we collected 21 maternal families from the area between the cities of Liberia and Bagaces in southern Guancaste. This is also a lowland area but represents a genetically distinct population from the other lowland population near sector Santa Rosa. The range of locations where families were collected represents the southernmost known populations of *Q. oleoides*. Acorns were germinated and seedlings were grown in plastic nursery bags with a 1:1 mix of sand and peat. Acorn diameter and length were measured on a sample of seeds

from a subset of the families. These measurements were used to calculate acorn volume while a calibration curve from a plot of other *Q. oleoides* acorn volume and mass measurements allowed us to estimate acorn mass. A T-test was used to test for a significant difference between upland and lowland acorn mass. We did not weigh the actual acorns that were germinated in the nursery. The seedling nursery, located near the lowland garden in sector Santa Elena of the ACG, was covered with 70% shade cloth. Seedlings were well watered and grew for 2-3 months prior to transplantation. Two weeks before transplantation, we removed the shade cloth and reduced watering to allow for acclimation to ambient solar and moisture conditions.

Two gardens were established in old pastures adjacent to current *Q. oleoides* stands. Existing pasture vegetation was cleared, fenced, and covered with black plastic sheeting in order to prevent competition of other vegetation with the seedlings. The lowland garden (280 meters above sea level) was located near the seedling nursery in sector Santa Elena (10°55’12’’N, 85°36’39’’W), and the upland garden (800 meters above sea level) was located near the entrance to Parque Nacional Rincón de la Vieja (10°46’23’’N, 8 5°21’03’’W) (Figure 3.2). The seedlings were arranged in a randomized block design over three blocks, and an equal number of seedlings per family were represented in each garden.

1668 seedlings were planted through 30cm diameter holes in the plastic sheet in October 2006 and censused at least twice per year over the next three years. Plant height, stem diameter, number of leaves, and length of longest leaf

were recorded at each census. After six months, the plastic sheeting was removed and the growth of pasture vegetation was controlled by periodic mowing.

Data Analysis

We fit a nominal logistic model using the program JMP (JMP, Version 7. SAS Institute Inc) to test for the effects of garden, population, and family on seedling survival. Garden, population and family nested within population were random effects in the model. This model was compared to all subset models using a likelihood ratio Chi-square test, and a likelihood ratio test also compared how each factor affected the fit of the model. To provide a comparison between the survival rate of garden seedlings and naturally occurring seedlings we monitored the fate of 258 tagged seedlings over one year.

To evaluate the effects of source population, location and their interactions on overall fitness, we used the recently developed maximum-likelihood approach called Aster Models (Shaw *et al.* 2008; Geyer *et al.* 2007) written in the R statistical language (R Development Core Team 2006). This method is an improvement over ANOVA methods because fitness components (for example survival and growth over multiple seasons and stages) are modeled with different statistical distributions and because it accounts for the dependence of fitness components expressed later in the life of the organism on those expressed earlier (Shaw *et al.* 2008).

Survival at each time period, modeled as a Bernoulli distribution, and final leaf number, modeled as a Poisson distribution were jointly analyzed for all analyses in Aster (Figure 3.3). Final leaf number was chosen as a surrogate measure of fitness because it is has been shown to be correlated with biomass (R^2=0.58, Figure 3.4) (unpublished data Cavender-Bares 2007). We fit multiple models of increasing complexity to the integrated survival and final leaf number data and compared them with likelihood-ratio tests. We compared how a model that included family (model 2) fit the data compared to a simpler model of just garden, block and population (model 1). We also compared the model that included family (model 2) to one that included a family x garden interaction (model 3). Evidence for local adaptation looks is provided by a significantly better fit to the data for a model containing a population x garden interaction (model 4) than a model without that interaction (model 1). Local adaptation is inferred if fitness of the home population is higher in the home site relative to its fitness elsewhere.

Results

The full logistic model fit the survival-only data better than any subset model, and the effect of garden was a significant factor in the model (effect likelihood ratio tests, $p<0.0001$). Survival was considerably reduced in the lowland garden relative to the upland garden, but mortality increased in each sampling period in both gardens (Figure 3.5). Three years after initial planting,

fewer than 40% of seedlings planted in the upland garden survived and fewer than 10% of seedlings planted in the lowland garden survived. A plot of the proportion of seedling survival per family in both gardens shows again that survival in each family was greater in the upland garden for both upland and lowland populations (Figure 3.6).

Mean acorn mass was highly variable among families (1.6 ±0.07 grams – 7.6 ±0.2 grams, Figure 3.7A). However, the difference in the average acorn mass between upland and lowland populations as not significant (Figure 3.7B). In the Aster analyses, we found that a model containing family fit the data better than a model without the factor of family (Table 3.1A). Also, a model containing a family x garden interaction fit the data better than without the interaction (Table 3.1B). In the test for local adaptation, the model containing a garden x population did not fit the data significantly better than the model without the garden x population interaction (Table 3.1C).

A plot of the combined survival and leaf number fitness estimates shows that fitness was higher for both populations in the upland garden after three years in 2009 (Figure 3.8). The mean for the lowland population (7.16) was greater than for the upland population (6.35) in the upland site as well as the lowland site (3.06 and 2.67 respectively). A comparison of Aster results over three sampling periods showed a trend toward a reduced difference in the mean fitness for each population in both gardens as the experiment progressed (Figure 3.9, shown without the third population for clarity).

Discussion

In this study, we hypothesized that differences in the abiotic conditions of the lowland and upland environments act to select against *Quercus oleoides* seedlings from the opposite environment. The results of our Aster analysis on the joint survival and leaf number data did not support this hypothesis. The model that included a population x garden interaction did not fit the data significantly better than a model without that interaction. We did however, discover an extremely large difference in overall survival between gardens, and we attribute this difference to the increased dry season severity of the lowland environment. Data from an observational experiment showed that there was no difference in the mortality of naturally occurring seedlings in the upland and lowland environments (Table 3.2). However, those seedlings were located under the forest canopy, and the gardens were located in adjacent pastures. The lowland pasture environment is considerably less hospitable than underneath the forest canopy (Appendix 2.1). To illustrate this further, data from an abandoned study (discontinued due to uncontrolled mortality) showed that supplemental dry season watering of seedlings in one lowland site led to an increased growth rate in those seedlings compared to the unwatered control (Appendix 2.2). This adds additional support to the interpretation that the lowland pasture environment caused the significantly higher mortality there.

The fitness of families from the lowland population was higher in the lowland environment. Paradoxically, that relationship was maintained in the upland environment, suggesting that lowland populations have an advantage in both environments. Since we did not have a third garden in the native environment of the 'other population', we cannot determine if the observed response in this population is similar to that seen in the lowland population. One interpretation of the consistently higher fitness of the lowland population is that the lowland populations have undergone selection for increased plasticity relative to the upland population, due to experiencing a more variable environment (de Jong 2005; Sultan 2000; Via *et al.* 1995). It is not clear if that has happened, however, especially when fitness in year three is compared to years one and two. The difference between the mean fitness of the upland and lowland populations in both environments decreased as time progressed (Figure 3.9).

Another possible interpretation for the consistently higher fitness of the lowland population is that unaccounted factors, such as maternal effects, caused lowland populations to have larger seed sizes. We did not include the seed mass data in the Aster analysis because measurements for all families were not taken. The measurements that we did take, however, indicate that there was no significant difference in the initial size of the seeds among the populations in this experiment. If the lowland population families had overall larger seeds, then the higher fitness of lowland individuals could be attributed to maternal effects rather than increased seedling performance (Bischoff *et al.* 2006). In at least two other

oak species, *Quercus suber* and *Quercus douglasii*, it has been shown that acorn size is larger in populations from drier habitats (Ramirez-Valiente *et al.* 2009; Rice *et al.* 1993). This subject deserves closer examination in our site in order to confidently compare studies. We chose to focus on achieving the desired amount of replication by planting seedlings into the gardens, but it is possible that the lack of signal for local adaptation may not be the same signal observed if seeds were planted directly in the gardens (Raabova *et al.* 2007). We did not see a significant difference in the size of the acorns from different populations, but the seedlings that germinate and grew in the nursery may have passed through a selective filter since the nursery was in the lowland environment.

It is not clear how long this advantage by the lowland population will last based on the three year trend reported here. The dominance by the lowland population may be a short term advantage that will disappear by the time these long lived trees reach reproductive maturity. Although we did not find evidence for maternal effects, we do not have a strong indication for increased plasticity either. Increased plasticity resulting from high environmental heterogeneity has been shown to be correlated with greater genetic diversity (Linhart & Grant 1996). In our earlier work on population genetic structure, we did not observe significantly greater genetic diversity (H_e) in the lowland population relative to the upland population (Chapter 1).

Our previous work on the genetic structure of *Q. oleoides* showed that genetic distance among populations was not correlated with geographic distance

or flowering time but one marker showed a significant correlation with the environment. Additionally, the amount of environmental variation between the lowland and upland sites is striking. The fact that we do not see local adaptation is surprising but is most likely due to sufficient long distance pollen dispersal among these sites as we reported in Chapter 2 (Hereford & Winn 2008). We have previously estimated average pollen dispersal distances to be quite limited, but rare long distance movement has so far prevented adaptive differentiation between the live oaks in these two sites. The observed differentiation at microsatellite loci must not be associated with traits under selection in these environments. Others have pointed out how poorly molecular markers are at indicating heritable variation in adaptive traits (McKay & Latta 2002) and examples exist in other species of *Quercus* where morphology remains consistent despite high variation in variation in molecular markers (Gonzalez-Rodriguez *et al.* 2005). In cases where populations are reproductively isolated, adaptive divergence, and even incipient speciation, is likely to occur. Lowry et al. showed that in *Mimulus guttatus*, coastal and inland populations selected against immigrants due to flowering time differences and adaptations to seasonal drought or coastal moisture (Lowry *et al.* 2008). We conclude that despite the steep environmental gradient among populations and large differences in survival between environments, sufficient gene flow has prevented local adaptation from occurring in *Q. oleoides* populations of Guanacaste, Costa Rica

Table 3.1 Results of likelihood ratio tests from Aster models

A) Model	Df	ModelDev	Df	Deviance	P(>|Chi|)
1	17	-87106			
2	62	-87269	45	163	>0.001*
B) Model	**Df**	**ModelDev**	**Df**	**Deviance**	**P(>|Chi|)**
2	62	-87269			
3	107	-87374	45	106	>0.001*
C) Model	**Df**	**ModelDev**	**Df**	**Deviance**	**P(>|Chi|)**
1	17	-87106			
4	19	-87110	2	4	0.1506

Model 1 (garden:block, population)
Model 2 (garden:block,population, family)
Model 3 (garden:block, population, family x garden)
Model 4 (garden:block, population, garden x population)

Table 3.2 Fate of 258 naturally occurring seedlings after one year

	lowland	**upland**
Not Found	25.68%	27.27%
Found Alive	63.51%	62.73%
Found Dead	5.41%	2.73%
ID tag only	5.41%	7.27%

Figure 3.1A. Comparison of monthly precipitation in lowland and upland environments of Guancaste, Costa Rica.

Figure 3.1B. Principal coordinates analysis using environmental data (temp, Rh, light, and soil moisture) collected from three upland sites (SC=sendero caballo, VJ=valle jabely, AO=agave oaks) and three lowland sites (FJ=finca jenny, CF=corta fuego, SE=santa elena). Arrows illustrate eigenvectors for the variables. PCA axis 1 is significantly correlated with elevation (R^2=83.2%, p=0.011).

Figure 3.1A.

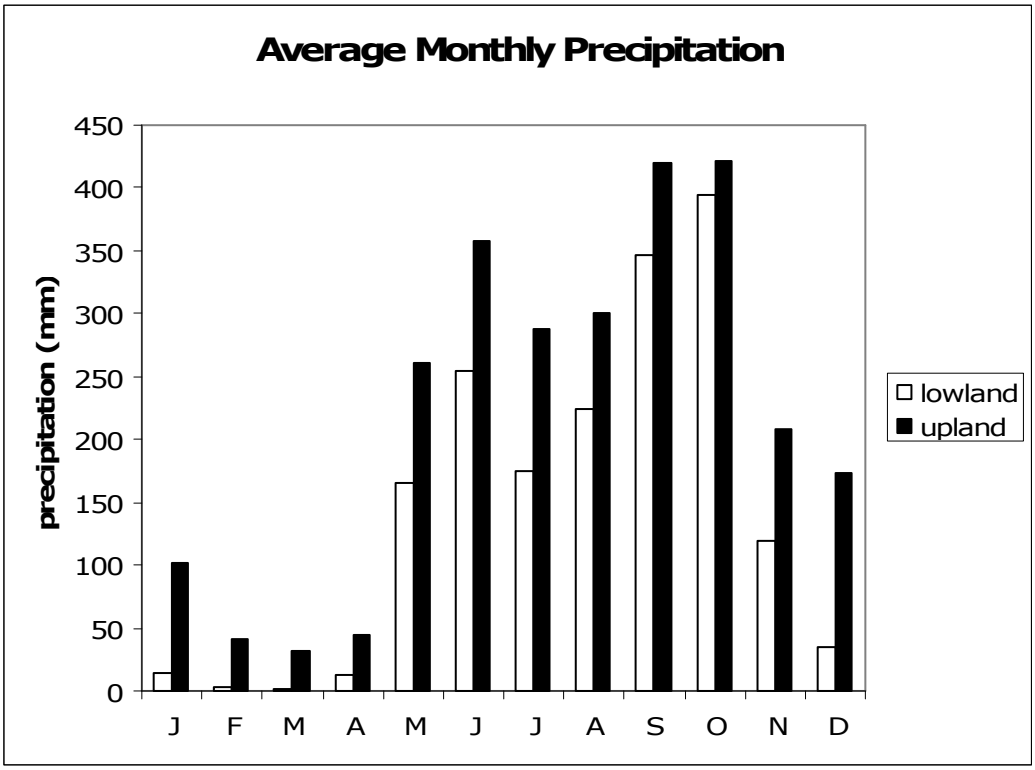


Figure 3.1B.

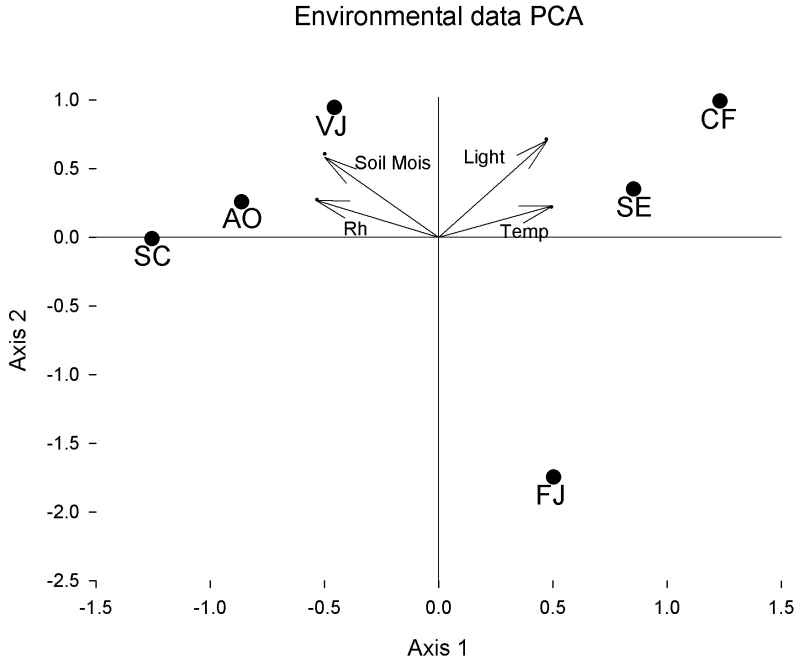


Figure 3.2. Map of northwest Costa Rica showing the location of the two gardens (stars) and family collection sites (polygons).

Figure 3.2.

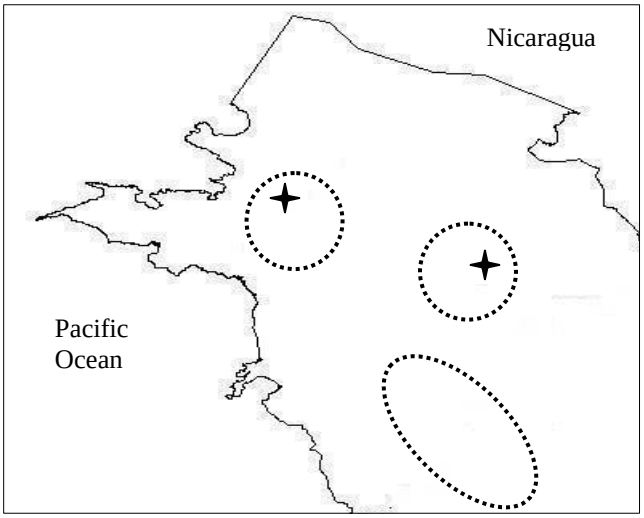


Figure 3.3 Diagrammatic representation of data collection and the statistical distributions used in the Aster method to estimate fitness (adapted from Shaw et al. 2008). Asterisk indicates point of transplanting from seedling nursery into one of two gardens.

Figure 3.3

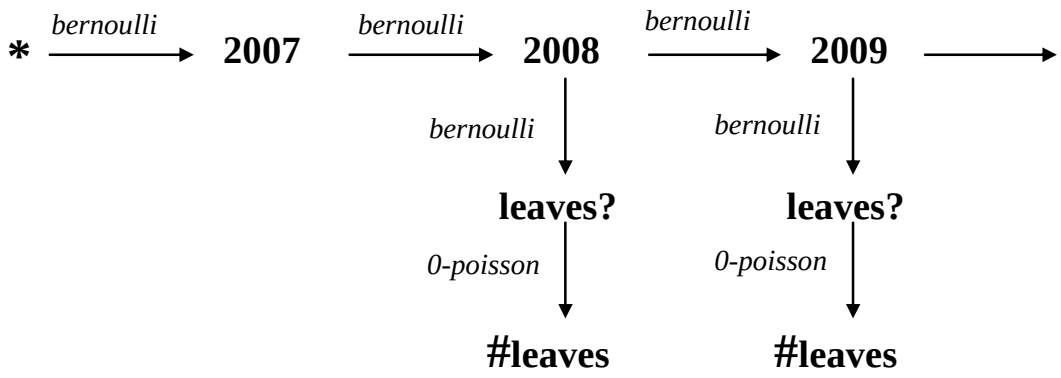

*
bernoulli
2007
bernoulli
2008
bernoulli
2009
bernoulli
leaves?
0-poisson
#leaves
bernoulli
leaves?
0-poisson
#leaves

Figure 3.4. Correlation between total leaf number and total biomass in *Quercus oleoides* seedlings (unpublished data from Cavender-Bares et al. 2007).

Figure 3.4.

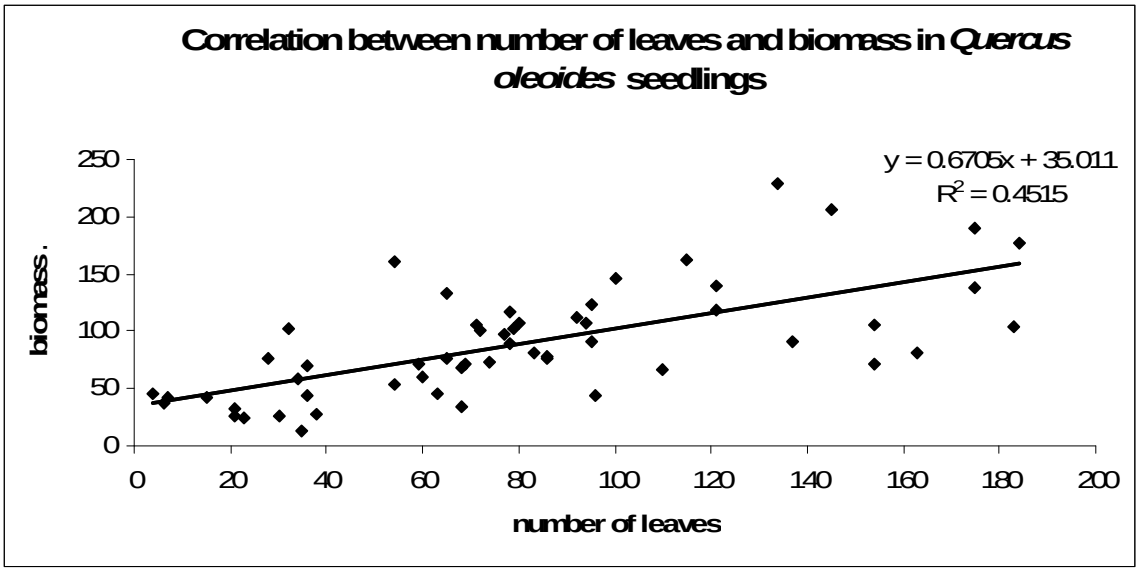

Correlation between number of leaves and biomass in *Quercus oleoides* seedlings
y = 0.6705x + 35.011
R² = 0.4515
biomass.
number of leaves
0
50
100
150
200
250
0
20
40
60
80
100
120
140
160
180
200

Figure 3.5. Seedling survival in upland and lowland gardens in January 2007, January 2008, and January 2009.

Figure 3.5

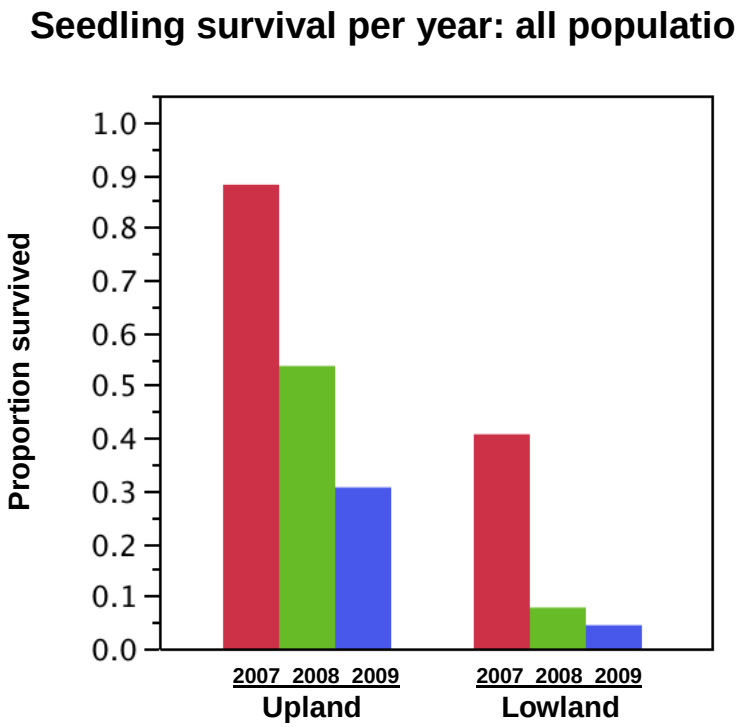

Seedling survival per year: all populations
Proportion survived
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
2007 2008 2009
Upland
2007 2008 2009
Lowland

Figure 3.6. Average survival of each family in both the lowland and upland gardens in 2007(A), 2008(B), and 2009(C). Upland families are solid shapes and lowland families are open.

Figure 3.6A.

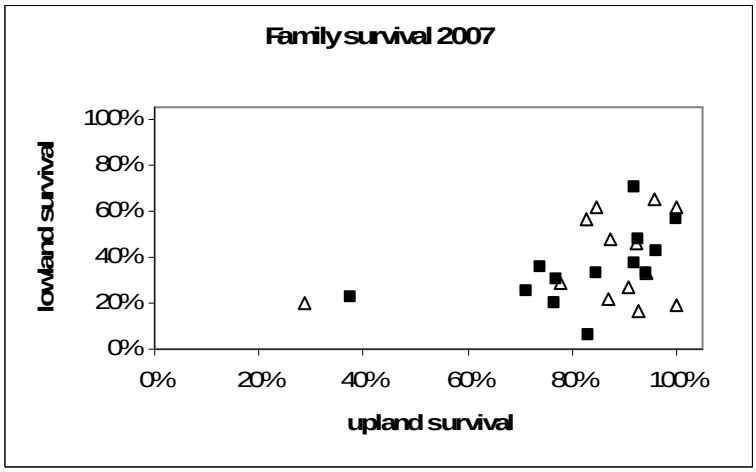


Figure 3.6B.

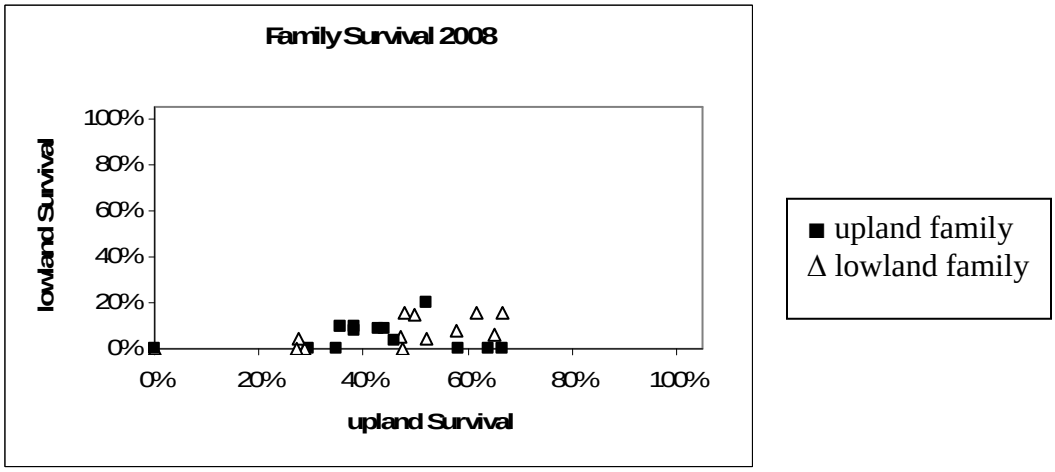


Figure 3.6C.

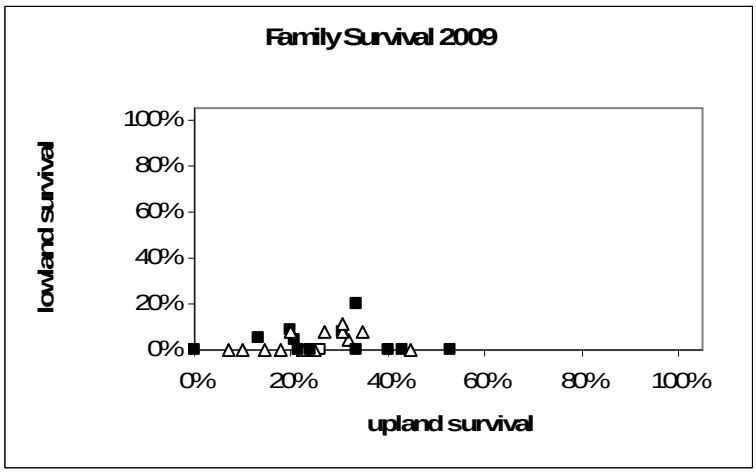


Figure 3.7A. Average acorn mass per family for a sample of acorns from some lowland (L), upland (U), and other (O) populations.

Figure 3.7B. Average seed mass per population weighted by the number of acorns measured per family. In both A and B, error bars are standard errors and mass was estimated from volume (((length+length+width)/6)^3*PI()*4/3) using a calibration curve (0.9846*volume 0.153, R^2=0.9).

Figure 3.7A

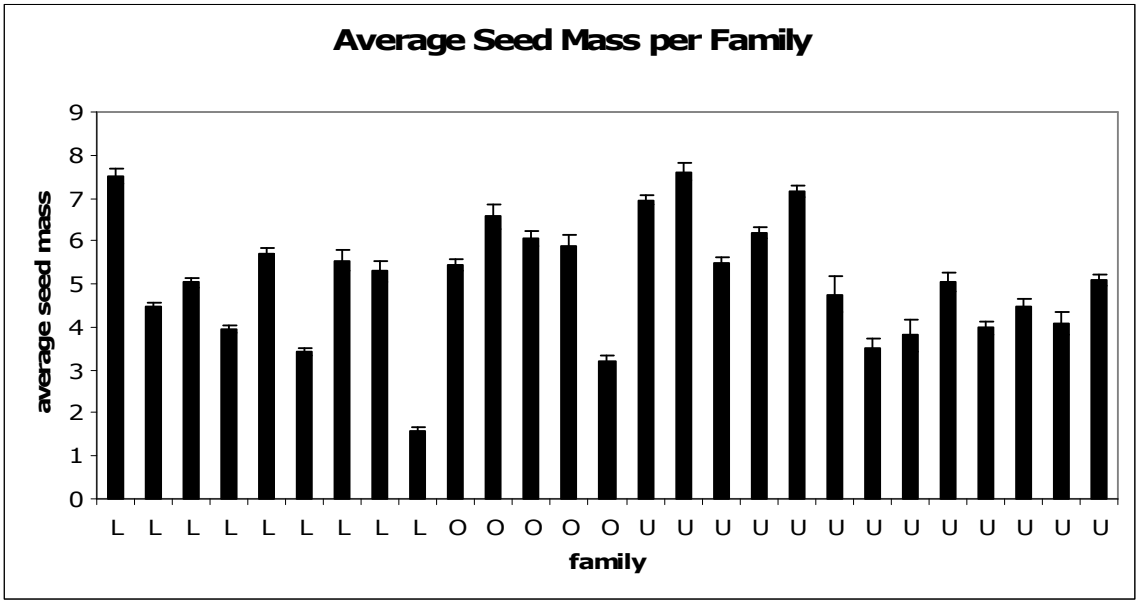


Figure 3.7B.

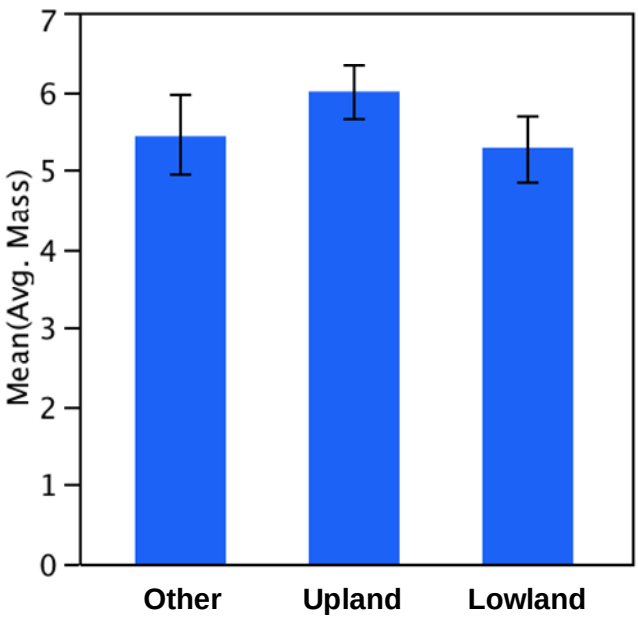


Figure 3.8. Plot of Aster fitness component (combined survival and number of leaves) averaged over three blocks for each population in the lowland and upland garden.

Figure 3.8.


Fitness across gardens
Aster fit (leaves)
10
9
8
7
6
5
4
3
2
1
0
lowland
upland
other pop
upland pop
lowland pop

Figure 3.9 Comparision of Aster fitness component (combined survival and number of leaves) at three time intervals for the lowland and upland populations in the two gardens. A) Fitness comparison in January 2007, B) Fitness comparison in January 2008, C) Fitness comparison in January 2009.

Figure 3.9A.

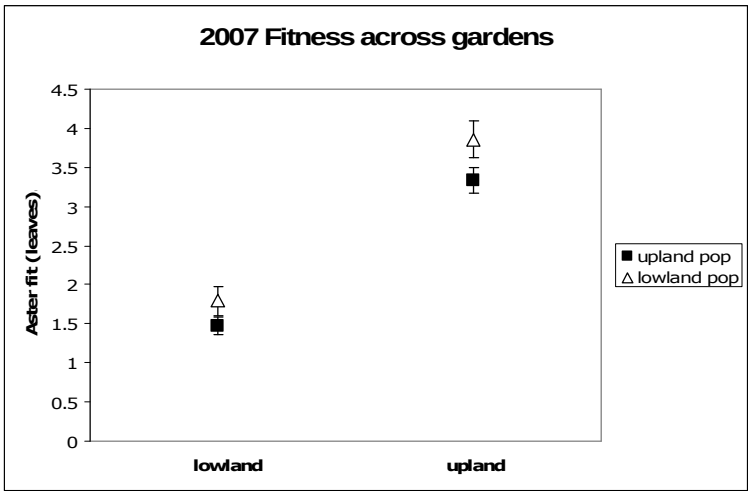
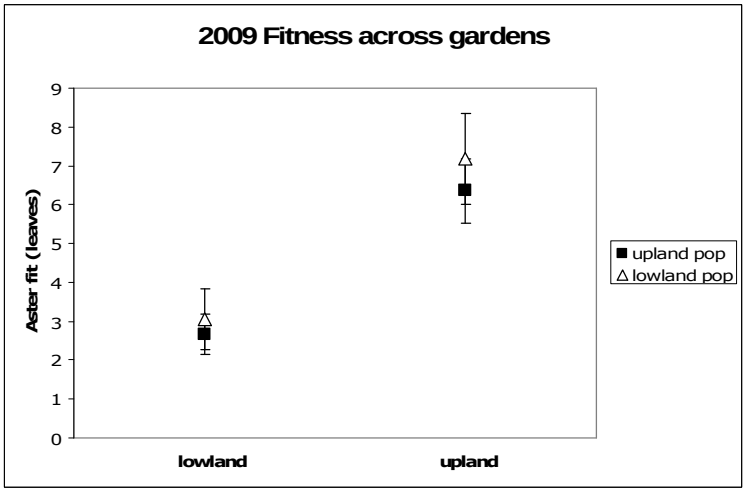


Figure 3.9B.

Figure 3.9C.



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Appendix 1

Population genetic distance matrix (Nei's genetic distance)

	FJ	SE	CF	SC	VJ	AO	PS	EH	LP	SJ	GY	PSE	RM
FJ	0.000												
SE	0.154	0.000											
CF	0.108	0.078	0.000										
SC	0.287	0.310	0.253	0.000									
VJ	0.188	0.198	0.182	0.184	0.000								
AO	0.197	0.172	0.167	0.137	0.113	0.000							
PS	0.150	0.105	0.140	0.292	0.188	0.138	0.000						
EH	0.185	0.261	0.286	0.362	0.316	0.326	0.290	0.000					
LP	0.235	0.177	0.163	0.281	0.167	0.176	0.254	0.287	0.000				
SJ	0.286	0.203	0.198	0.289	0.137	0.173	0.257	0.376	0.048	0.000			
GY	0.287	0.247	0.244	0.283	0.138	0.186	0.263	0.380	0.082	0.086	0.000		
PSE	0.181	0.168	0.180	0.327	0.115	0.187	0.187	0.284	0.057	0.067	0.067	0.000	
RM	0.206	0.199	0.212	0.327	0.152	0.187	0.219	0.322	0.078	0.086	0.121	0.053	0.000

Population geographic distance matrix (km)

	FJ	SE	CF	SC	VJ	AO	PS	EH	LP	SJ	GY	PSE	RM
FJ	0.00												
SE	7.07	0.00											
CF	3.46	4.58	0.00										
SC	25.27	31.58	28.73	0.00									
VJ	23.83	30.18	27.28	1.46	0.00								
AO	24.21	30.50	27.67	1.10	0.64	0.00							
PS	7.78	4.90	7.43	29.06	27.72	27.96	0.00						
EH	13.52	10.17	13.39	31.11	29.91	30.03	5.97	0.00					
LP	19.20	25.94	22.59	7.13	5.77	6.37	24.18	27.33	0.00				
SJ	31.43	38.07	34.84	7.31	8.40	8.34	35.93	38.30	12.26	0.00			
GY	48.32	55.33	51.48	26.62	27.55	27.60	53.99	57.05	29.84	19.37	0.00		
PSE	12.06	10.15	9.01	36.44	34.98	35.44	14.95	20.26	29.76	41.90	56.98	0.00	
RM	17.92	11.33	15.91	39.73	38.45	38.64	11.05	9.35	35.19	46.75	65.01	18.61	0.00

Population environmental distance matrix (normalised elevation, vegetation, and climate)

	FJ	SE	CF	SC	VJ	AO	PS	EH	LP	SJ	GY	PSE	RM
FJ	0.000												
SE	0.271	0.000											
CF	0.271	0.000	0.000										
SC	3.952	3.799	3.799	0.000									
VJ	3.639	3.462	3.462	0.778	0.000								
AO	4.099	3.935	3.935	0.241	0.778	0.000							
PS	0.679	0.567	0.567	3.436	3.055	3.577	0.000						
EH	0.679	0.567	0.567	3.436	3.055	3.577	0.000	0.000					
LP	1.830	1.658	1.658	2.184	1.809	2.313	1.269	1.269	0.000				
SJ	2.771	2.624	2.624	1.192	1.010	1.352	2.246	2.246	1.014	0.000			
GY	0.978	1.077	1.077	4.868	4.494	4.999	1.490	1.490	2.704	3.690	0.000		
PSE	0.392	0.121	0.121	3.736	3.388	3.866	0.554	0.554	1.591	2.565	1.140	0.000	
RM	0.151	0.121	0.121	3.866	3.539	4.006	0.604	0.604	1.731	2.687	1.025	0.241	0.000

Proportion of male flower cooccurrence matrix (eq. 1, below diagonal) and overlap matrix in proportion of male flowering (eq. 2, above diagonal).

	FJ	SE	CF	SC	VJ	AO	PS	SJ	GY	RM
FJ		0.266	0.132	0.099	0.296	0.419	0.031	0.081	0.100	0.598
SE	0.250		0.066	0.090	0.338	0.445	0.176	0.144	0.359	0.306
CF	0.095	0.129		0.227	0.169	0.209	0.782	0.650	0.110	0.454
SC	0.100	0.134	0.223		0.749	0.663	0.480	0.189	0.408	0.230
VJ	0.277	0.173	0.152	0.632		0.851	0.270	0.207	0.096	0.198
AO	0.339	0.248	0.214	0.584	0.729		0.404	0.218	0.237	0.274
PS	0.050	0.191	0.581	0.398	0.190	0.286		0.747	0.392	0.453
SJ	0.100	0.144	0.503	0.128	0.114	0.125	0.617		0.152	0.441
GY	0.150	0.378	0.095	0.294	0.089	0.202	0.314	0.167		0.513
RM	0.336	0.331	0.354	0.248	0.188	0.207	0.385	0.342	0.374	

Appendix 2: Abiotic conditions across forest-pasture transition and between upland and lowland

Figure 1.
Abiotic differences in adjacent forest and pasture ecosystems. A) Daily integrated PAR across the forest-pasture ecotone during the wet and dry season averaged for six sites. The x-axis indicates the distance from the edge (0) into the forest or the pasture. B) Estimated values of volumetric soil water content (θ) to 30 cm depth averaged for 10 m and 20 m into the forest or the pasture for the three upland (800m) and three lowland (300m) sites. θ measurements were made using time domain reflectometry and calibrated with the equation θ = (67.345*x) - 149.74 for highlands and θ = (32.643*x)-67.678 for lowlands, where x is flight time of the electromagnetic pulse in arbitrary units, following Cavender-Bares and Holbrook (2001). C) Seasonal high and low daily means for temperature and (D) relative humidity at 20 m into the forest or pasture for the same sites. For B-D, significant differences between means in the forest and the pasture, based on two-way ANOVA with habitat and site as main effects, are indicated as follows: *** p< 0.001, ** P< 0.01, * P< 0.05.

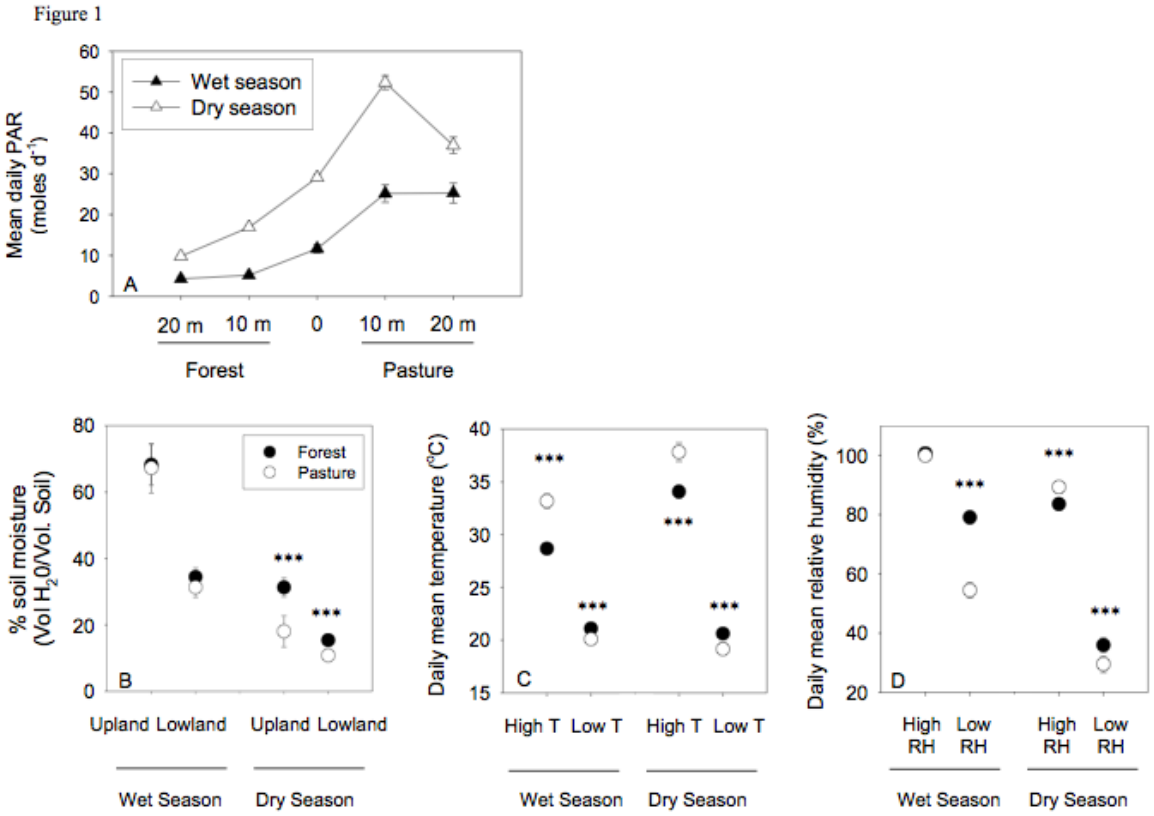

Figure 2. A full factorial experiment with water, shade, and grass competition was no completed due to unaccounted for mortality. Below are data from the one site where the experiment was completely destroyed. Releative growth rate was calculated after one year using a calibration curve from Quercus virginiana (Cavender-Bares 2004). In A) Water was added weekly over the dry season to the experimental group (1). B) 70% shade cloth was placed over the experimental group (1). C) Vegetation was removed around plants in the experimental group (1).