

CLONALITY AND GENETIC DIVERSITY IN
POLYGONELLA MYRIOPHYLLA, A LAKE WALES RIDGE
ENDEMIC PLANT

by

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ABSTRACT

Although capable of sexual reproduction, many plants also rely heavily on clonal reproduction. The formation of multiple, physiologically-independent units with the same genotype has important implications for spatial genetic structure and genetic diversity in these plants. The endangered scrub-dwelling perennial, *Polygonella myriophylla* is known to reproduce both sexually and clonally but no study to date has been able to investigate the spatial genetic patterns that occur in this species. I use microsatellite markers to investigate questions about clonal structure and genetic diversity in five populations of *P. myriophylla* and address some of the implications of my findings for conservation of this species: Overall, I find that 57% of sampled clusters of *P. myriophylla* are composed of a single genet (genetic individual) with multiple physiological units (ramets) while the remainder are made up of two or more genets. I found differences in both clonal reproduction and genetic diversity among populations. I also found evidence of limited gene flow even over small spatial scales (less than 10 km) and for at least 4 genetic clusters occurring within the species range. Despite high levels of genetic diversity overall, there is evidence of reduced genetic diversity in two populations. My results suggest that high levels of clonality may be important in maintaining genetic diversity in *P. myriophylla*. I also provide evidence that dirt roadsides may not represent a refuge for this species.

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LIST OF ACRONYMS

A	Number of alleles
A_R	Allelic richness
CRC	Lake Wales Ridge National Wildlife Refuge – Carter Creek Tract
CTC A	Allen Davis Broussard Catfish Creek State Park population A
CTC B	Allen Davis Broussard Catfish Creek State Park population B
Dr. P	Dr. Phillips, FL
H_E	Expected heterozygosity
H_O	Observed heterozygosity
HWE	Hardy Weinberg Equilibrium
K	Number of genetic clusters
LE	Linkage equilibrium
LP	Lake Placid, FL
MLG	Multilocus genotype
MP2	Macroplot 2 (at CRC)

MP3	Macroplot 3 (at CRC)
MP4	Macroplot 4 (at CRC)
n	Number of samples
N_{CL}	Number of clusters
N_R	Number of ramets
P_C	Proportion of clones
PID	Probability of identity
Road	Roadside (at CRC)

CHAPTER 1: INTRODUCTION

Although sexual reproduction predominates in vertebrates, asexual or clonal reproduction is extremely common in many other taxa, including fungi, protists, bacteria, some invertebrates, and plants (Anderson & Kohn 1995; Ayre & Hughes 2000; Klimes 1997; Simon et al. 1999). Clonal reproduction can take a variety of forms but all have the same basic result: creation of a new physiologically independent unit with an identical genotype to the parent. In plants, one of the most common forms of clonality is vegetative reproduction, in which new physiological individuals are formed without spores or gametes (Klimes 1997). Although many discussions of sexual and clonal reproduction treat it as an either-or decision (Doncaster et al. 2000; Hamilton et al. 1990), many organisms utilize both (Green & Noakes 1995; Handel 1985). This results in a host of interesting interactions, with clonal reproduction driving changes in sexual reproduction and vice versa (Hamilton et al. 1987; Handel 1985). Clonal reproduction changes the distribution and diversity of genotypes in a population, ultimately impacting the genetic diversity, population structure, and potentially the very survival of an organism (Aarssen 2008; Honnay & Bossuyt 2005; Pan & Price 2001). The particular impacts of clonality on these characteristics of a species are highly dependent on both the demographic characteristics of that species and the manner in which it employs clonal reproduction (Engelstadter 2008; Judson 1997; Liu et al. 2009). Understanding the implications of clonal reproduction becomes particularly important when the species in question is threatened or endangered, and clonality can serve as a “double-edged sword” when conservation is a priority.

Clonality-induced changes in genetic structure may cause a loss of genetic diversity and amplify the impact of reduced population size. For example, when used extensively, clonal reproduction can result in clusters of identical genotypes, especially when mobility of the organisms is limited (Araki et

al. 2009; Charpentier 2001; Handel 1985; Herben & Novoplansky 2008). These clusters then promote inbreeding, because the most readily available mates are also the most closely related. When self-fertilization is possible, this clustering of closely related genotypes may result in high levels of inbreeding leading to a rapid reduction of genetic diversity within the population (Leiss et al. 2009; Thompson et al. 2008). The negative interaction between self-fertilization and clonal reproduction is expected to create selection pressure favoring self-incompatibility. However, this phenomenon has not been conclusively demonstrated (Jacquemyn & Honnay 2008; Vallejo-Marin & O'Brien 2007). When self-fertilization is not possible, genotypic clustering can inhibit outcrossing and reduce effective population size, ultimately reducing genetic diversity (Liao et al. 2009). Another way in which clonal reproduction results in the loss of genetic diversity occurs when relatively few genotypes are able to outcompete others, resulting in a dramatic reduction in the effective population size (Lhuillier et al. 2006; Mock et al. 2008; Noel et al. 2007). In the most extreme circumstances, a single genotype may dominate a habitat patch, completely eliminating the opportunity for outcrossing and potentially halting sexual reproduction altogether (Lhuillier et al. 2006).

Interestingly, clonal reproduction can also forestall the loss of genetic diversity in some circumstances and allow more time for management strategies to be devised. By creating additional physiologically independent units the chance of death for any individual genotype is reduced (Pan & Price 2001; Seligman & Henkin 2003). This results in maintenance of alleles in the population for longer periods of time and reduces the impact of stochastic events on the genetic makeup of a population. Clonality also permits those alleles to be retained in the population long-term, even if sexual reproduction is impossible due to poor conditions or a lack of mates (Aarssen 2008; Dorken & Eckert 2001; Honnay & Bossuyt 2005). These effects are especially important in small species in

which each physiological individual is relatively short-lived (Aarssen 2008). In the case of plants, clonal reproduction also enables a larger and more prominent floral display, which may attract pollinators and increase opportunities for outcrossing (Charpentier 2001; Leiss et al. 2009).

Plants of all types frequently employ clonal reproduction but it is especially common in small angiosperms (Aarssen 2008; Crepet & Niklas 2009). As a result, a single genetic individual, or genet, may be made up of many physiologically independent units, or ramets. Mobility of genotypes is extremely limited in plants and the spatial genetic structure created by patterns of ramets and genets can play a major role in the larger patterns of genetic diversity (Reisch et al. 2007). The type of clonal reproduction is a key factor in the spatial genetic structure of plants, and may be thought of as occurring along a gradient (Doust 1981; Herben & Novoplansky 2008). At one end of the gradient is phalanx type reproduction, where new ramets are formed immediately next to previously existing ramets. This creates a clustered pattern of genets in the population with all ramets from a single genet being in close proximity to one another. At the opposite end of the spectrum is guerilla type clonal recruitment, in which new ramets are dispersed over considerable distances from the previously existing ramets. When guerilla recruitment predominates, individual ramets from a single genet will be found widely scattered and interspersed, with little or no clustering by genotype (Doust 1981).

In addition to the impacts of vegetative recruitment, genetic structure in plants is also affected by historical patterns of colonization and long distance gene flow. In species where long-distance gene flow is rare and areas of suitable habitat are naturally fragmented, local adaptation to environmental conditions is expected to be important and species may possess characteristics that prevent the loss of genetic diversity even with limited gene flow (Hamrick 2004). This may result in the formation of

multiple genetic clusters, with little gene flow across areas of unsuitable habitat and high gene flow within patches of suitable habitat. Alternately, a more continuous pattern of increasing genetic isolation with increasing geographic distance between populations, or isolation by distance (IBD) may be seen (Wright 1943). As a result, it is important to understand both fine scale patterns of genetic diversity derived from clonal and sexual reproduction, and larger scale patterns derived from habitat fragmentation and long-distance gene flow. For example, working with the endangered plant *Centaurea horrida*, Mameli et al. (2008) found evidence for two distinct genetic clusters, correlated with a 30 km gap in suitable habitat, a result that indicated that the two clusters needed to be considered separately for conservation planning.

The Lake Wales Ridge is an elevated area consisting primarily of scrub habitat that was the shoreline of Florida during glacial minima of the Pleistocene. The region is of particular interest for conservation planners because it is home to many rare and endemic species, including one species of bird, one mammal, many other vertebrates and invertebrates, and more than 21 species of plants. Moreover, at least 80% of the xeric upland habitat that characterizes the ridge, and upon which these species depend, had been lost by 2007 (Turner et al. 2006; Weekley et al. 2008). Genetic studies of scrub species on the LWR have demonstrated a high degree of variation in reproductive patterns, genetic diversity, and population structure. One plant, *Ziziphus velata*, has been found to occur almost exclusively as uniclinal populations (Godt et al. 1997; Weekley et al. 2002), while other plants show genetic diversity ranging from relatively low to extremely high (Dolan et al. 1999; Evans et al. 2000; McDonald & Hamrick 1996). Additionally, some species show almost no population structure, with more than 95% of variation being found within populations, while others are highly genetically structured, with more than 50% of variation being found among populations. Studies of the Florida scrub-jay and the sand skink both show evidence of multiple genetic clusters within the

Lake Wales Ridge, and this clustering is combined with a general pattern of isolation by distance in the case of the skinks (Coulon et al. 2008; Richmond et al. 2009).

My study focused on *Polygonella myriophylla*, a clonal, allelopathic shrub currently listed as endangered at both the federal and state level (Turner et al. 2006; Weekley et al. 2008). Although it is most closely associated with the Lake Wales Ridge, and has even been used to help define the boundaries of that ridge in some areas (Weekley et al. 2008), it is also found on two other Central Florida ridges, though only on the portions of those ridges closest to the Lake Wales Ridge. *Polygonella myriophylla* is considered an important component of healthy scrub habitat and may play an important role in moderating the effects of fire by impacting the growth and distribution of scrub vegetation through its allelopathic effects (Weidenhamer & Romeo 1989, 2004).

Polygonella myriophylla typically grows in distinct clusters that are surrounded by a margin of bare sand, resulting from the allelopathic chemicals released by the plant. Attempted sexual reproduction is common and plants produce flowers year-round, but seeds and seedlings are rarely observed (Quintana-Ascencio et al. 2008). A recent study found that less than 1% of flowers result in seeds, and seedlings occur almost exclusively after disturbance events (Quintana-Ascencio et al. 2008). Most reproduction in this species appears to occur clonally, through adventitious rooting of branches running near the ground. Over time, these rooted branches may disconnect from the parent plant and as a result each genetically unique individual (genet) may be comprised of many independently rooted branches (ramets; Fig. 1). Because multiple seedlings may sprout in close proximity to one another (Quintana-Ascencio et al. 2008) it is impossible to determine from a visual inspection whether all ramets within a single cluster of *P. myriophylla* belong to the same genet or multiple genets. To circumvent this problem in the absence of molecular markers, a previous field

study defined a “functional genet” as a ramet or group of ramets of *P. myriophylla* separated from other ramets by more than 30 cm (Quintana-Ascencio et al. 2008). This study is the first to evaluate the utility of this operational definition through the use of genetic markers.

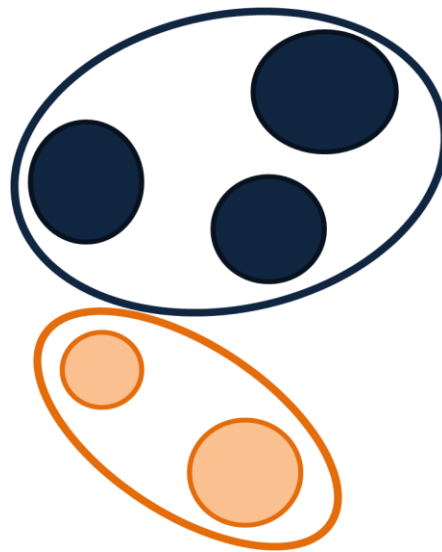


Figure 1: Diagram illustrating the distinction between ramet and genet. Each filled circle represents a single, independently rooted branch, or ramet. Each empty circle surrounds all ramets of a single genetic individual, or genet. Colors correspond to unique genets. This figure shows five ramets that together make up two genets.

Polygonella myriophylla generally occurs in two types of habitat: scrub typical of its native range, and along the side of dirt roads that cut through scrub habitat (Quintana-Ascencio et al. 2008). These two habitats are subjected to different disturbance regimes. Scrub patches typically experience periodic fires (every 15-100 years; Meyers 1990), currently in the form of prescribed burns, after which *P. myriophylla* must sprout from seed. In contrast to the episodic disturbance occurring in scrub habitats, *P. myriophylla* growing in roadside patches experience more continuous disturbance in form of continuously shifting sand, which alters the likelihood of ramet formation through adventitious rooting and increases variability in growth rate and other demographic features

(Quintana-Ascencio et al. 2008). *P. myriophylla* growing along roadsides may also be less likely to experience fire which may alter the rate at which new genets (seedlings) can enter the population. Additionally, the scrub habitats in which *P. myriophylla* occur differ in patch size, isolation from other scrub patches, and time since fire. Small habitat patch size and isolation of a habitat patch may reduce genetic diversity of populations over time as a result of genetic drift and increased inbreeding (Aguilar et al. 2008). A previous study using allozymes identified high levels of genetic diversity in *P. myriophylla* but was not able to evaluate clonal structure and thus could not consider the impact of clonal reproduction on population genetic structure and genetic diversity (Lewis & Crawford 1995).

I used microsatellite markers to investigate clonal reproduction and spatial patterns of genetic diversity in *Polygonella myriophylla*. The questions I sought to address are: 1) Does the distribution of ramets and genets suggest a phalanx or guerilla type of vegetative recruitment strategy? 2) Does the frequency of clonal reproduction differ between sites or between habitat types within sites? 3) Do some populations contain more genetic variation than others? 4) Is there an effect of isolation by distance within the species range? 5) Are there multiple genetic clusters, and if so, how are they distributed?

CHAPTER 2: MATERIALS AND METHODS

Sample collection

I selected four locations where *P. myriophylla* is known to occur, representing most of the north-south extent of the species range (Figs. 2 and 3; Turner et al. 2006). The northernmost site, Dr. Phillips, FL (Dr. P) is located on the Mt. Dora ridge. The other three sites, Allen Davis Broussard Catfish Creek State Park (CTC); Lake Wales Ridge National Wildlife Refuge Carter Creek Tract (CRC); and Lake Placid, FL (LP) are located on the Lake Wales ridge. I sampled from multiple areas at CRC and CTC (Fig. 3). At CRC, I collected samples within the scrub habitat in three macroplots that were managed with different strategies: burned, roller chopped, and undisturbed. I also sampled along the dirt roads dividing the CRC macroplots. Because these areas were not separated by unsuitable habitat and occurred over less than 1 km I consider them one population, except when considering differences between road and scrub plants. At CTC, I collected samples in the scrub and along dirt roads from two areas separated by approximately 7 km of mostly unsuitable habitat. These two areas are identified as CTC A and CTC B and are considered separate populations unless otherwise noted.

Within each site, I selected clusters of *P. myriophylla* to sample haphazardly. Each new cluster was picked by moving away from the previous cluster until another cluster was seen. In instances where another cluster was immediately visible from the cluster being sampled I ensured that sampled clusters were separated by at least 5 meters of bare ground or other vegetation. The location of each cluster was recorded using a Tremble GPS unit with submeter accuracy. For all clusters larger than 30 cm in diameter and with more than one ramet (86% of sampled clusters), I collected leaves from two or more ramets and recorded the distance(s) between the samples in centimeters using a tape

measure (Fig. 4). I collected a total of 291 ramets from 153 clusters of *P. myriophylla*. The total number of samples and clusters for each site is given in Tables 1 and 2. Leaf samples consisted of approximately 40-60 young leaves, placed in a plastic bag containing Drierite dessicant (Drierite co.).

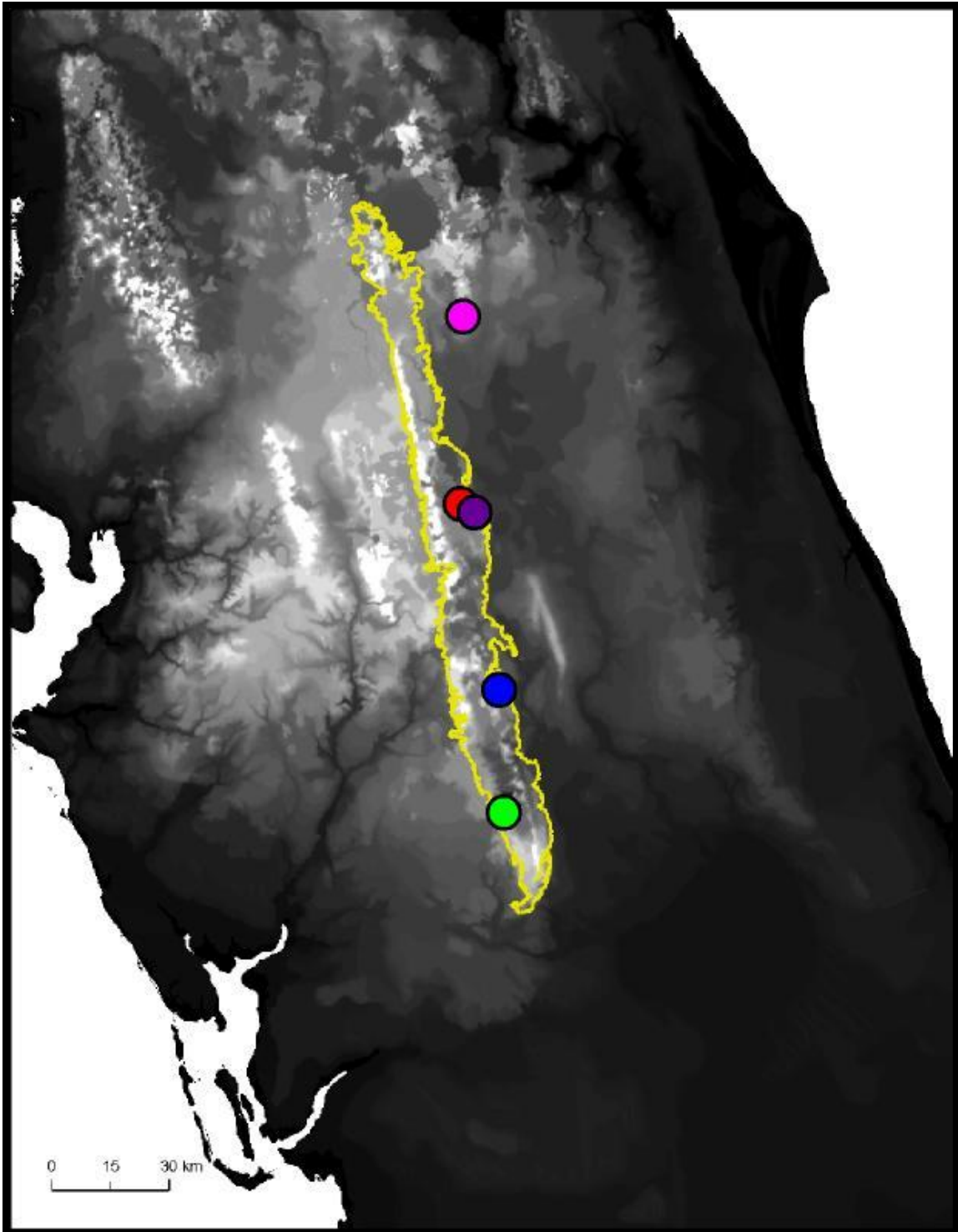


Figure 2: Topographic map of the central Florida showing collection sites for *Polygonella myriophylla*. Yellow outline denotes the boundary of the Lakes Wales Ridge and circles indicate collection sites. Pink = Dr. P, Red = CTC A, Purple = CTC B, Blue = CRC, Green = LP.

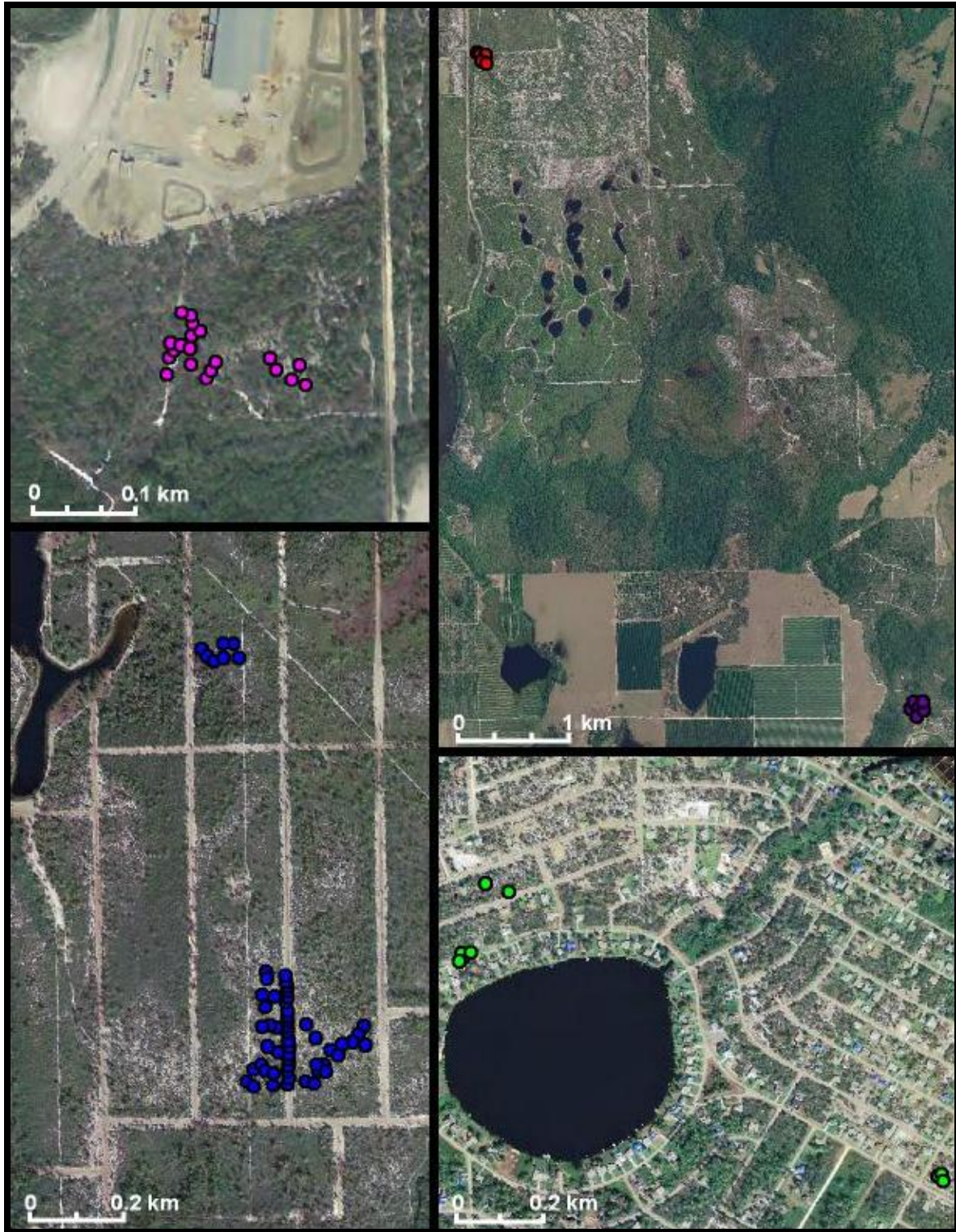


Figure 3: Satellite images showing collection localities for *Polygonella myriophylla*. Top left – Dr. P (pink). Top right – CTC (red = CTC A, purple = CTC B). Bottom left – CRC (blue). Bottom right – LP (green).



Figure 4: Diagram of collection method used to sample clusters of *Polygonella myriophylla*. The pink circle indicates the outermost extent of the sampled cluster. Yellow circles are the branches where the leaf samples were taken. Green line is the distance between samples.

Table 1: Number of clusters (N_{Cl}) and ramets (N_R) for all populations of *P. myriophylla* collected for this study. Dr. P = Dr. Phillips, CTC = Catfish Creek, CRC = Carter Creek, LP = Lake Placid

Population	Dr. P	CTC A	CTC B	CRC	LP	Total
N_R	44	38	42	151	16	291
N_{Cl}	22	24	22	75	10	153

Table 2: Number of clusters (N_{Cl}) and ramets (N_R) for all subpopulations of *P. myriophylla* collected at the CRC site. MP2 = Macroplot 2, MP3 = Macroplot 3, MP4 = Macroplot 4, Road = Roadside between macroplots

Subpopulation	MP2	MP3	MP4	Road	Total
N_R	14	44	34	59	151
N_{Cl}	7	22	16	30	75

To extract DNA, I pulverized the leaf tissue by placing approximately 20-25 dried leaves in a 2.0mL screw-top microcentrifuge tube with 10-15 2.0 mm zirconia beads, then shaking the tube for 3 minutes in the MiniBeadBeater 16 (BioSpec Products, Inc.). After the cell walls were disrupted I used a standard DNA extraction protocol designed for plant tissue (Qiagen Plant DNeasy Mini Kit). I verified successful DNA extraction by visualizing a portion of the extract on a 1% agarose gel or by quantifying the extracted product using a Nanodrop spectrophotometer (Thermo Scientific, Inc.).

Marker development and genotyping

I used a microsatellite isolation protocol based on the method used in Edwards et al. (2007), summarized here. I began by cutting the total genomic DNA into shorter pieces using the *Sau3AI* restriction enzyme and then ligating the fragments produced to the *Sau3AI* linkers. I enriched the library for CA repeats by hybridizing the fragments with a biotinylated (CA)₁₅ probe. I captured the target fragments using Streptavidin MagneSphere Paramagnetic Particles (Promega) and washed the beads with a series of buffers. The stringency, or quantity of nonmicrosatellite-containing DNA washed off of the beads, was adjusted by changing the concentration and temperature of the buffer used. After the washes were completed, I stripped the probe from the DNA and cloned the enriched product using a PCR cloning kit (Qiagen). To screen for microsatellites I performed two PCRs on each cloned sample, one containing only the M13F and T7 primers, and one containing M13F, T7, and a (CA)₁₅ repeat primer (Degner et al. 2009). Samples containing a repeat region produce a shorter band or smear in the (CA)₁₅ PCR relative to the two-primer PCR. I screened 768 samples and found 115 (15%) in which the PCR results indicated potential presence of a repeat. I sent the PCR product from all positive samples to the Nevada Genomics Center for sequencing in both directions on an ABI 3730 DNA analyzer. I screened all sequences for repeat regions,

compared repeat-containing sequences and removed identical sequences. Through this screening procedure, I identified 29 unique repeat-containing regions.

I designated the 29 repeat-containing sequences as Pmyr_001-Pmyr_029 and designed 1-4 primer pairs for each of the potential loci using the program Primer3 v0.4.0 (Rozen & Skaletsky 2000). I attached an M13 tag to the end of all forward primers (Schuelke 2000). I tested primer pairs for amplification by running PCR reactions for each pair using the *P. myriophylla* sample from which the primers were derived as a positive control and one additional *P. myriophylla* sample. If primer pairs successfully amplified these two individuals, I tested them with an additional subset of *P. myriophylla* samples from across the species range. In this second test, I used a PCR technique that attaches a fluorescently labeled M13 primer to the amplified microsatellite, allowing products to be visualized on a CEQ 8000 DNA analyzer (Beckman-Coulter; Schuelke 2000). I checked a portion of PCR product from the labeled PCRs on a 2% agarose gel and purified the remaining product using the manufacturers recommended procedure before loading the plate onto the CEQ. I used the Beckman-Coulter CEQ software to analyze readouts and to look for topologies consistent with microsatellites. I scored loci with appropriate topologies for the sample set to check for polymorphism, or variation among individuals within the sample.

After polymorphic ($n = 4$) loci were identified, I obtained direct labeled forward primers for those loci to facilitate genotyping. PCR conditions for amplification of polymorphic loci can be found in Table 3. To test for cross-species amplification I attempted to amplify polymorphic loci in three additional *Polygonella* species that occur in the same scrub habitat as *P. myriophylla* and represent each of three major phylogenetic clades found within the genus *Polygonella* (Lewis & Crawford 1995): *P. basiramia*, *P. robusta*, and *P. polygama*.

Table 3: Primers, repeat length, and PCR conditions for all loci used in this study.

Locus	Primers (5' – 3')	Repeat	MgCl₂	Temp
Pmyr_001	F - AAT CCT GAA TTC CCT TCA ATAT R - TGG TAT GTG TTT CTG CTG AG	CA ₁₀	2.0	55
Pmyr_015	F - GGC ACA TGT GAC CTA AAT CT R - ACA CTA TAA GCT TTG TTA CAC C	CA ₁₁ CA ₁₀	3.4	56
Pmyr_020	F - ACT TGC CCT GCA CTA ACT C R - GTT TCA CCT CCT ACA TCA GCA AAG	CT ₁₃ CT ₅ CA ₇	3.4	60
Pmyr_023	F - TGT CGA GGA AAC TAG ACG TT R - GTT TGC TAA AGG TCT GGA TGT GAA	CA ₂₂	3.4	56

Clonal structure and diversity

To evaluate clonal structure and to remove duplicate genotypes from population genetic analyses, I compared all multilocus genotypes (MLGs) using the program GenClone v2.0 (Arnaud-Haond & Belkhir 2007), which determines the number of MLGs present and also identifies genotypes that may represent mistakes in the genotyping process or somatic mutations. I removed duplicate MLGs and analyzed those remaining using the program Gimlet v1.3.3 (Valiere 2002) to identify the probability of identity (PID) for each genotype. The PID is the probability that two full siblings would share a MLG, given the frequency of each allele in the population being measured (Valiere 2002). I calculated PIDs at the population level. I considered any shared MLG with a $PID \leq 0.05$ to be evidence that the samples sharing the MLG represented ramets of a single genet.

For each population, I calculated the proportion of clones (P_C) as the number of clusters where all samples shared a MLG divided by the total number of clusters sampled. I compared the distances

between ramets for clusters that contained one genet to those that contained multiple genets using a one-tailed t-test to determine if the average size of clusters containing multiple genets was larger than those containing a single genet.

Hardy-Weinberg equilibrium and linkage

To evaluate loci for deviations from Hardy-Weinberg equilibrium (HWE) and test for linkage disequilibrium after duplicate genotypes were removed, I used the program Arlequin 3.11 (Excoffier et al. 2005). To look for the presence of null alleles or genotyping errors due to large allele dropout or stutter, I used the program Microchecker v2.2.3 (Van Oosterhout et al. 2004).

Genetic diversity and population genetic structure

I performed an AMOVA to determine the distribution of variation within and among populations using Arlequin 3.11 (Excoffier et al. 2005) and estimated values of F_{ST} (Weir & Cockerham 1984) for all pairwise populations using the program GenePop v4.0 (Raymond & Rousset 1995; Rousset 2008). I also calculated expected and observed heterozygosity (H_E and H_O , respectively), number of alleles (A) and allelic richness (A_R) for each population. To check for evidence of isolation by distance I used the program IBDWS v3.1.6 (Jensen et al. 2005) to perform a Mantel test.

To determine the number of genetic clusters (K) present in the *P. myriophylla* species range I used STRUCTURE v2.3.1 (Pritchard et al. 2000) and the R package Geneland (Guillot et al. 2005; Guillot et al. 2008). For STRUCTURE, I used both an admixture model with no location data and an admixture model that included information about sampling localities. Both models assumed correlated allele frequencies between populations. The inclusion of location data allows STRUCTURE to better predict the number of clusters for small data sets and is not prone to the

identification of spurious clusters (Hubisz et al. 2009). All STRUCTURE analyses were run for $K = 1$ through $K = 10$, with a burnin of 1.5×10^5 generations and run time of 3.5×10^5 generations. The ΔK criterion (Evanno et al. 2005), which measures the change in the likelihood value between different values of K , was used to select the most likely number of clusters under each model.

For Geneland, I performed 10 independent runs and chose the best run based on the mean posterior density. For each run, I used the spatial model and assumed uncorrelated allele frequencies. As with STRUCTURE, I evaluated the number of clusters for $K = 1$ through $K = 10$. As recommended in the user manual for Geneland, I set the maximum rate of the Poisson process to equal the number of individuals (genets; $n = 191$) and the maximum number of nuclei in the free Voronoi tessellation to three times that number. Each run had 3×10^5 MCMC iterations, recorded every 50 iterations, and had a post process burnin of 2000 saved iterations.

CHAPTER 3: RESULTS

Marker development

Of the 29 loci initially screened, nine successfully amplified and exhibited microsatellite-like topologies. Four of the nine frequently amplified more than the expected two alleles (*P. myriophylla* is diploid; Lewis & Crawford 1995). This amplification of additional products occurred for all primers developed for these loci, so the loci were discarded. Of the remaining five loci, one locus was fixed in all populations and four were polymorphic. These four loci were retained for this study and were polymorphic in all populations. Locus Pmyr_001 had the lowest diversity, with 6 alleles across the species range and 2-6 alleles per population. Pmyr_023 had the highest diversity: 35 alleles total, 7-31 per population (Table 4). Because a four locus genotype was required to adequately identify clones based on PID, I discarded all samples for which all loci had not amplified after a minimum of three attempts to do so. The total number of ramets for which I obtained a complete MLG is given in Table 4.

In all but one population, a MLG with four loci was sufficient to obtain a PID value of ≤ 0.05 . The exception to this was the Dr. P population, where approximately half of the samples with 4 locus MLGs had population-level PID values between 0.05 and 0.1. Because the Dr. P site did not show significant deviations from HWE after removal of duplicate MLGs I considered it unlikely that there were a high number of undetected clones. Additionally, there were only 7 clusters where both branches sampled shared a genotype, so the expected number of misidentified clones within the population is less than 1. As a result I retained all samples from the Dr. P site for this study.

After Bonferoni correction ($P = 0.0025$) linkage was detected for only a single pair of loci in one population and deviations from HWE were significant for only one locus x population combination (Pmyr_023 in CRC). This did not exceed the number of significant results expected under neutral conditions. Null alleles were detected in two loci in one population (Pmyr_020 and Pmyr_023 in CRC) and one locus in each of two populations (Pmyr_020 in CTC B, Pmyr_023 in Dr. P) but the levels identified were less than 10%. I did not find evidence of genotyping errors due to large allele dropout or stutter for any locus in any population using Micro-checker. Because no locus or population consistently deviated from neutral expectations, all loci and populations were retained for use in this study.

Clonal distribution

Across all populations the P_c was 57% and within populations it ranged from 37-66% (Table 4). The proportion was lowest at the Dr. P site and highest in CRC. When only two ramets were sampled from a cluster ($n = 105$) the P_c was 58%. When more than 2 ramets were sampled from a single cluster of *P. myriophylla* ($n = 5$) the P_c was 40%, two genets were detected 40% of the time and more than 2 genets were detected 20% of the time. The average diameter of clusters with a single genet was 165.3 cm and the average diameter of clusters containing multiple genets was 264.9 cm and multigenet clusters were significantly larger ($P < 0.001$). In one instance, the same genet was detected in two adjacent clusters separated by just over 5 meters.

Genetic diversity

Expected heterozygosity (H_E) ranged from 0.41 to 0.95 for each locus and 0.61 to 0.79 for each population (Table 4). Observed heterozygosity (H_O) was generally similar to or lower than H_E . The largest difference between the two values was found in the CTC A site, where overall H_O was

significantly lower than H_E ($P = 0.0001$). H_O was also lower than H_E in the CTC B site, but the difference was comparable to other sites and was not significant ($P = 0.05$). Both H_O and H_E for Dr. P were lower than values for any other site. Allelic richness (A_R), rarified to 12 diploid genets, showed a similar pattern, with values for Dr. P being the lowest across all loci (Table 4).

Table 4: Population genetic information for all loci and populations of *P. myriophylla* used in this study. A_T = total number of alleles, A = alleles per population, A_R = allelic richness (rarified to 12 individuals), H_O = observed heterozygosity, H_E = expected heterozygosity, N_R = Number of ramets, N_G = number of genets, N_{Cl} = Number of clusters, P_C = proportion of clones.

Locus	A_T		Dr. P	CTC A	CTC B	CRC	LP
All Loci		N_R	40	31	41	131	15
		N_G	33	23	32	91	12
		N_{Cl}	19	11	19	56	5
		P_C	0.37	0.64	0.47	0.66	0.60
Pmyr_001	6	A	2	3	4	6	3
		A_R	2.0	3.0	3.4	4.6	3
		H_O	0.45	0.43	0.53	0.69	0.42
		H_E	0.41	0.52	0.59	0.67	0.45
Pmyr_015	29	A	9	12	13	20	11
		A_R	7.0	8.3	9.1	11.8	11
		H_O	0.64	0.65	0.81	0.92	0.92
		H_E	0.74	0.79	0.86	0.92	0.88
Pmyr_020	17	A	8	7	9	12	8
		A_R	5.6	6.4	7.2	7.9	8
		H_O	0.67	0.74	0.78	0.71	0.50
		H_E	0.65	0.81	0.83	0.81	0.80
Pmyr_023	35	A	7	12	15	31	12
		A_R	5.8	10.0	10.9	15.2	12
		H_O	0.67	0.70	0.81	0.82	1.00
		H_E	0.76	0.86	0.90	0.95	0.87
Overall		H_O	0.61	0.63	0.73	0.79	0.71
		H_E	0.64	0.75	0.80	0.84	0.75

Population genetic structure

I found that 7.8% of the total variation was distributed among populations (global F_{ST} 0.078) and 92.2% was within populations. My estimates of pairwise F_{ST} between populations ranged from 0.028 - 0.120 (Table 5) which represents low to moderate population differentiation (Wright 1978). I found the highest population differentiation ($F_{ST} = 0.120$) between Dr. P and the two CTC populations and the lowest ($F_{ST} = 0.028$) between Dr. P and LP. I found no effect of isolation by distance using the program IBDWS ($P = 0.534$).

Table 5: Geographic (km; above diagonal) and genetic (F_{ST} ; below diagonal) distances for all populations of *Polygonella myriophylla* used in this study.

Population	Dr. P	CTC A	CTC B	CRC	LP
Dr. P	-	46	52	96	126
CTC A	0.120	-	7	50	80
CTC B	0.116	0.072	-	44	74
CRC	0.091	0.080	0.056	-	30
LP	0.081	0.028	0.045	0.056	-

The number of clusters recovered using STRUCTURE varied depending on the model employed. For the admixture model with no location prior, I found the most likely number of clusters based on the ΔK criterion to be 7, which also had the highest likelihood value (-3401.244 ± 28.551). An evaluation of the bar plot for $K = 7$ (data not shown) showed that this result was biologically unrealistic and no clear clusters were being formed.

Using the admixture model with a location prior, I identified the most likely number of clusters based on the ΔK criterion to be two and there was an additional peak at $K = 4$, which also had the highest likelihood value (likelihood for $K = 2$ is -3432.284 ± 1.354 , likelihood for $K = 4$ is -3243.792 ± 13.329). The two clusters identified were Dr. P and a cluster containing all Lake Wales ridge populations (Fig. 5). Because STRUCTURE primarily identifies the highest level of population structure and the ΔK and likelihood measures gave conflicting results I reran the analysis with the Dr. P population removed. This additional run also used the admixture model with a location prior and the most likely K based on both the ΔK and likelihood criteria was $K = 3$ (likelihood -2864 ± 7.673). The three clusters identified correspond to CTC A, CTC B, and CRC (Fig. 5). The LP samples do not show a strong association with any cluster but are most closely associated with the CRC cluster.

Results from Geneland are similar to those from STRUCTURE. With all sites included, Geneland identified $K = 4$ as the most likely number of clusters and all ten runs showed the same 4 clusters. The clusters identified in the best run are shown in Figure 6 and correspond to Dr. P, CTC A + LP, CTC B, and CRC. The only meaningful difference between the STRUCTURE and Geneland results is the assignment of the LP samples to the same cluster as CTC A by Geneland and the same cluster as CRC by STRUCTURE.

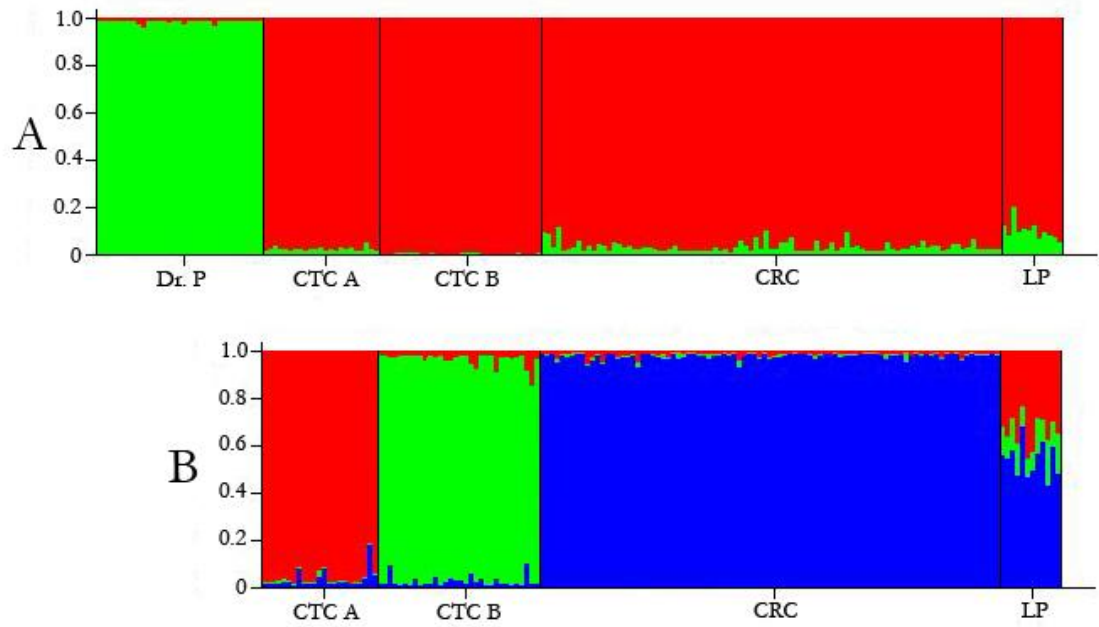


Figure 5: Bar graphs from STRUCTURE analyses showing A) $K=2$ clusters for the species range as a whole and B) $K=3$ clusters for the Lake Wales Ridge only. The Y axis shows values for the proportion of each genotype attributed to a particular cluster and the X axis shows population names.

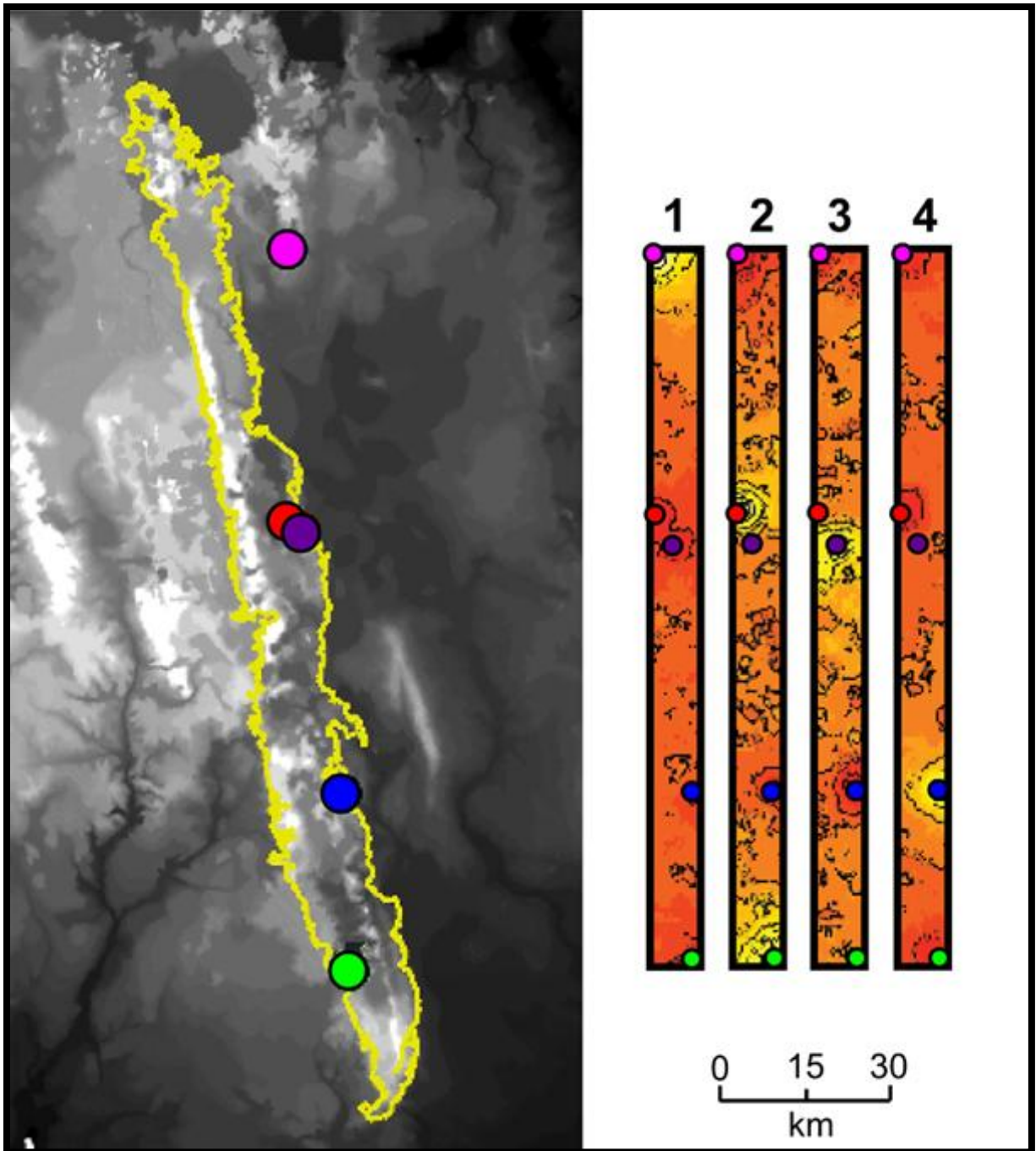


Figure 6: Genetic clusters inferred using Geneland. Left - topographic map of the Lake Wales Ridge (outlined in yellow) showing collection sites for this study. Right - Geneland heat maps (right) showing posterior probabilities of belonging to one of four inferred clusters. Yellow indicates areas with a high probability of genotypes from that area belonging to a genetic cluster, orange and red represent lower probabilities of belonging to that cluster. Colored dots correspond to collection sites. Pink = Dr. P, Red = CTC A, Purple = CTC B, Blue = CRC, Green = LP.

CHAPTER 4: DISCUSSION

Marker development

Marker development was extremely difficult for *P. myriophylla*. Sequences of positive PCR products identified many sections of highly similar DNA. Additionally, during the process of screening potential loci, nearly half had to be rejected due to the presence of more than 2 alleles in multiple samples, regardless of the primer pair used to amplify the locus. These problems were unique to the loci in question, as the same samples did not have extra alleles when amplified for other loci.

Although *P. myriophylla* is currently diploid, these two pieces of evidence point toward a history of extensive gene or genome duplication, which may make this species of interest for future studies of genome evolution.

Clonal diversity

There is extensive clonal reproduction in *P. myriophylla*, with more than half of the sampled clusters of this species containing only a single genet. However, the proportion of clones varies widely between sites and the factors influencing the proportion of clones could not be identified in this study. Interestingly, the lowest proportion of clones was found at the Dr. P site, which showed less overall genetic diversity, even though the number of genets sampled in that population was the second highest in this study. This decrease in clonal reproduction may be the cause of the genetic depression observed in that population, as more genetic individuals die because they are represented by only a few branches and are more prone to loss in stochastic events. Alternately, the genetic depression may be unlinked to the proportion of clones and may result from another process.

Of *P. myriophylla* clusters where more than 2 samples were collected, two were found to represent only a single genet, two contained two genets, and only one had more than 2 genets detected (5 ramets sampled, 4 genets detected). These results (40% one genet, 60% more than 1 genet) are similar to those found using only two samples per cluster (58% one genet, 42% more than 1 genet) and suggest that my within-cluster sampling was adequate to estimate general patterns of clonal recruitment in *P. myriophylla*. Though there are certainly instances where some genets within a cluster were not detected in this study, the ability to detect additional genets with increased sampling effort must be weighed against the loss in sampling efficiency. A 50% increase in sampling effort would likely only have detected 1-5 additional genets per population, which would be unlikely to have a significant impact on my analyses.

The average size of clusters that contained a single genet was significantly smaller than the average size of clusters containing more than one genet. This pattern was also consistent within populations (data not shown). It is unclear whether the larger clusters with multiple genets represent places where multiple, previously distinct clusters grew together over time, or habitat patches where multiple seedlings began growing in close proximity. Further study will be necessary to answer this question.

As expected, given that clonal recruitment in *P. myriophylla* occurs primarily through adventitious rooting, the spatial distribution of genets reflects a phalanx-type recruitment process in which new clones are produced by branches rooting and separating from the parent plant. I found only one instance of possible clonal recruitment at a distance more than 5 m, in which the same MLG was found at two consecutive sampling points. Based on the PID values recovered for the CTC A population (average PID = 0.024), it would not be unexpected to find one instance of samples that

share a MLG as a result of being siblings rather than being members of the same genet. Thus, this may represent either clonal reproduction over a longer than expected distance or two closely related genets occurring in close proximity to one another.

The phalanx type recruitment that occurs in *P. myriophylla* is likely both a help and a hindrance in the maintenance of genetic diversity within a population. Because the species is capable on self-fertilization, large clusters made up of multiple ramets of the same genet have the potential to increase the opportunity for inbreeding and may reduce the availability of outcrossed pollen. However, the shifting sand and harsh environment within the scrub habitat favored by this species makes the ability to produce multiple physiologically independent units important for the long term survival of a genet (Quintana-Ascencio et al. 2008). This effect may be enough to offset the negative repercussions of inbreeding.

Genetic diversity

Overall levels of genetic diversity are very high in *Polygonella myriophylla*, especially in light of its status as a clonal, self-fertilizing, endangered species with a highly restricted range. This pattern was noted during marker development when only one monomorphic locus was discovered and all other loci were highly polymorphic. All populations contain private alleles, and all loci have some alleles that are shared across all populations. The highest incidence of private alleles occurs in Carter Creek (CRC), which has 18 private alleles, and the lowest is Dr. Phillips (Dr. P), which has only 1 private allele. Private alleles were found for all loci except Pmyr_001.

The highest levels of genetic diversity are found at the CRC site. Of 87 alleles identified in this study across all loci and populations, 70 (80%) are found in CRC, which also has the highest allelic

richness and highest heterozygosity of the populations studied. Allelic richness and heterozygosity values are also high for the Catfish Creek B (CTC B) and Lake Placid (LP) sites. The high level of diversity found at the LP site is particularly interesting, because very few samples were collected there (15 ramets, 12 genets) largely due to difficulty in finding *P. myriophylla*. My results suggest that the LP population was once much larger and has only recently experienced population declines.

Only two populations, Dr. P and CTC A, show evidence of a reduced heterozygosity. In the case of Dr. P, both H_O and H_E were low relative to other populations, which I hypothesize is a result of long-term isolation of that population from other populations of *P. myriophylla* as a result of its location on the Mt Dora ridge. The situation in CTC appears to be more complex. The division of the two populations collected in the Catfish Creek preserve by STRUCTURE and Geneland, along with the relatively high F_{ST} value found between them, suggests that there may be restriction of gene flow even over short distances in this species. The two CTC populations also show different levels of heterozygosity, which may be linked to demographic differences between the populations. At CTC A, most plants grow on the edges of dirt roadways and there are very few plants present in the scrub. In contrast, at CTC B, most plants grow in the scrub habitat and only a few are closely associated with roadways. As a result, there is likely little gene flow between *P. myriophylla* growing along roadsides and those growing in the scrub habitat at CTC. At CRC, plants grow in both the scrub and roadside in close proximity to one another, which should allow for frequent gene flow. There is no reduction in heterozygosity for roadside samples at CRC. I hypothesize that roadside habitat is acting as a sink for this species, and that in the absence of scrub populations as a source of new genetic material roadside populations become genetically depauperate. A previous study suggested there is higher mortality and greater demographic variability in roadside habitats which may contribute to increased inbreeding and relatively rapid loss of genets in the roadside (Quintana-

Ascencio et al. 2008). This is in contrast to a previous hypothesis that suggested that dirt roadsides might be acting as a refuge for scrub species that depend on open patches of habitat in order to grow (Petru & Menges 2004; Quintana-Ascencio et al. 2007).

The high level of genetic diversity found in this study is similar to the results found in a previous study that used allozymes to examine genetic diversity in 11 *Polygonella* species. Lewis and Crawford (1995) found higher genetic diversity in endemic *Polygonella* species (including *P. myriophylla*, *P. basiramia*, *P. macrophylla*, and *P. parksii*) than in their more widespread congeners (*P. americana*, *P. articulata*, *P. gracilis*, *P. polygama*, *P. robusta* and *P. fimbriata*). Although 15 years have passed between these studies and results from allozyme and microsatellite based studies are not directly comparable, my results also show high levels of genetic diversity in *P. myriophylla* and suggest that, in general, genetic diversity is being retained over short time periods in this species.

Population genetic structure

In general, my estimates of F_{ST} show low to moderate population differentiation, and the global F_{ST} of 0.078 suggests moderate differentiation across the species range. Only LP has pairwise F_{ST} values that qualify as low. The low estimates of F_{ST} may result from the low sample size for the LP population and likely represent an underestimate of actual population differentiation. There is clear evidence of restricted gene flow between populations even when separated by less than 10 km (CTC A to CTC B, 7 km, $F_{ST} = 0.072$). Despite spatial clustering of clones and restricted gene flow between populations, I did not find evidence of a link between geographic and genetic distance at the level of the species range. It is likely that these populations of *P. myriophylla* are not in migration-drift equilibrium, as a result of long-term retention of alleles through clonal recruitment and very

low rates of sexual reproduction. Because opportunities for dispersal are closely tied to sexual reproduction in plants, low rates of sexual reproduction will also limit dispersal.

The STRUCTURE results are dependent on the model selected. Runs that did not include location priors found the best evidence for 7 clusters, but this did not produce a biologically relevant result.

A species range level analysis that included the location prior found the best evidence for 2 clusters: one including only the Dr. P site located on the Mt Dora Ridge, and one containing all 4 sites (CTC A, CTC B, CRC, and LP) located on the Lake Wales Ridge. This result is biologically reasonable, given that populations on different ridges have likely been separated for a considerable time and ridges are separated by areas of habitat that are unsuitable for scrub species (Weekley et al. 2008).

The presence of an additional peak in the ΔK values at $K = 4$ suggested an additional level of population structure, therefore I analyzed the data for the Lake Wales Ridge sites only. This analysis found that $K = 3$ based on both ΔK and likelihood criteria. The three clusters identified were CTC A, CTC B, and CRC. The LP samples did not clearly associate with any cluster, but there was not sufficient support to assign them to their own cluster, likely because of the low sample size for that site ($N_G = 12$).

The addition of location priors to STRUCTURE is relatively recent, but initial testing suggests it is a robust procedure, which improves the analysis without the risk of identifying spurious clusters on the basis of poor location information. The use of location priors is indicated when the data set is small (in sample size or in number of loci) and when the location data can be reasonably assumed to add information (Hubisz et al. 2009). My data set for *P. myriophylla* meets both of these criteria, with four loci and F_{ST} values that indicate restricted gene flow between locations. Additionally, STRUCTURE reports the effect of the locations priors on the model outcome through the

parameter 'r', and values less than 1 indicate that the model is not being overly influenced by location data (Hubisz et al. 2009). Because location priors are indicated for this dataset, values of r were low (avg r = 0.34), and I did not find biologically realistic clustering using the model without location data, I consider the results from the location prior model to be more reliable.

My Geneland results support the results from STRUCTURE; both the hierarchical analysis used to assign 4 clusters and the decision to use the location prior. Although the two programs are similar, they use different algorithms to assign clusters and have different methods of incorporating spatial information into the analysis. The results of both programs suggest that genetic structure in *P. myriophylla* is weak, but present. The only significant difference between the results of the two programs is the cluster to which the LP samples are assigned. This difference is most likely due to a lack of information in the LP samples. Only 12 distinct genotypes were collected from LP due to the small size of the population there. The heterozygosity and allelic richness of that site suggest that the LP population was once much larger and is not adequately characterized by the sample collected in the now suburban landscape.

CHAPTER 5: IMPLICATIONS

Clonal reproduction and estimates of population size

The results of this study confirm the importance of using genetic markers in order to obtain reliable estimates of population size and structure in clonal plants. For *P. myriophylla*, estimates of the number of genets based on numbers of rooted branches would tend to dramatically overestimate the population size, since each genet may have multiple ramets, while those based on the number of clusters of plants would underestimate it. The previous working definition of a genet is not an adequate descriptor of the distribution of ramets and genets in this species. In many cases I identified multiple genets within clusters of *P. myriophylla*, which would not have been predicted based on the working definition. In general, smaller clusters are more likely to be made up of a single genet of *P. myriophylla* and larger clusters are more likely to have multiple genets, but I could not identify a specific cutoff of distance between clusters or cluster size that could be used for a working definition.

My results also indicate that there is a need to assess the frequency of clonality and clonal structure independently for each population. Across the species range as a whole I found approximately 1.35 times as many genets as clusters of *P. myriophylla*. The ratio of clusters to genets varied with the population sampled, however, from as low as 1.25 (in CRC) and as high as 1.57 (in Dr. P). Although these differences might not be critical in a more widespread species they are very important in an endangered species where each genet is relatively more valuable in maintaining diversity. These differences among populations should be taken into account when determining management strategies for *P. myriophylla*.

Genetic diversity

The CRC population of *P. myriophylla* is the most genetically diverse of those I sampled and may be the largest extant population. This site should be given high priority in any management plan for this species and may also be a good source population if any attempts are to be made to repopulate areas from which *P. myriophylla* has been extirpated. In particular, although the CRC site is already a protected area, my results suggest that protection of *P. myriophylla* may need to be given a higher priority in management decisions than other species that occur there. Special care should be taken to preserve genetic diversity in this population of *P. myriophylla* because diversity lost from CRC cannot be reintroduced from other portions of the species range. CRC also has the highest proportion of clones out of all populations included in this study. This may be driven by the high genetic diversity, with the healthiest plants being capable of producing multiple ramets, or may be driving the high genetic diversity by keeping rare alleles in the population for longer periods of time. Alternatively, high clonality and high genetic diversity may both be linked to some other factor not measured in this study. Because clonality can be observed in “real time” but changes in genetic diversity will lag behind, the proportion of clones may prove to be a useful proxy for genetic diversity in this species.

There is a significant reduction in H_O compared to H_E in the CTC A population, in which plants are found primarily along the roadside, that is not seen in either the nearby CTC B population (in which plants are found primarily in the scrub), or in plants collected along the roadside in CRC (where plants grow in both the scrub and roadside). Because H_E is not reduced in CTC A compared to CTC B, the discrepancy between H_O and H_E is likely due to increased inbreeding occurring in the roadside habitat. This suggests that roadside populations of *P. myriophylla* may ultimately be acting as genetic sinks, unable to maintain the high levels of genetic diversity normally found in this species without

regular gene flow from scrub plants. This potential role of the roadside populations as genetic sinks needs to be further investigated if roadside plants are to be included in any management plan for this species.

Reduced heterozygosity is also found in the Dr. P population, the only population studied that occurs outside of the Lake Wales Ridge. Unlike CTC A, H_E is also reduced at Dr. P compared to all other populations studied. In fact, the Dr. P population has the lowest H_O , H_E , and A_R of all sites studied. This affects the results of the clone analysis by driving PID values up from an average of 0.0182 in other populations to an average of 0.0473 in Dr. P. These results suggest that the Dr. P population has experienced multiple generations of low population size, most likely combined with very low gene flow with other populations. The lack of private alleles in Dr. P, despite a relatively high sample size, suggests that the loss of alleles through genetic drift has outpaced the generation of new alleles via mutation in this population. From a conservation standpoint, the Dr. P population seems to offer little in terms of genetic diversity; however, it represents a natural experiment of the impacts of low population size in this species. Further work investigating whether genetic diversity continues to decline in this population and the rate at which it is being lost could be of considerable use in creating a management plan for *P. myriophylla*.

The LP population of *P. myriophylla* provides evidence of the lag that exists between changes in population size and structure and changes in genetic markers. Genetic diversity at LP, as measured by heterozygosity and allelic richness, is second only to CRC, but the LP population is rapidly dwindling and may become extinct within the next few years as a result of development and fire suppression (pers. comm., Quintana-Ascencio). This extinction will likely occur long before evidence of a declining population numbers can be expected to show up in genetic analyses. The

clonal reproduction and long lifespan of *P. myriophylla* reduce the genetic impact of small populations and buffer the plant against stochastic events but cannot stave off the complete loss of habitat occurring in this region.

Population structure

Despite the low number of populations and loci used in this study, I was able to detect moderate genetic structure. The most obvious divergence is found between the populations on the Lakes Wales Ridge and the Dr. P site on the Mount Dora ridge. However, this divergence appears to be driven by reduced genetic diversity of the Dr. P site, as the Dr. P population contains only a single private allele. If possible, additional populations on the Mount Dora ridge should be sampled for comparison to the Lake Wales Ridge sites. This will allow us to determine whether the detected divergence is between the two ridges, or is unique to the Dr. P site.

Although there is population differentiation, even at relatively small spatial scales, most variation in *P. myriophylla* is found within populations. This is useful from a conservation perspective as it suggests that this species is well adapted to a fragmented distribution. Additionally, conservation efforts targeted at a few large populations should permit most of the genetic diversity currently present in the species to be retained. The Carter Creek and Catfish Creek populations are good candidates for such efforts.

Management and planning

My results for *P. myriophylla* should be useful for informing management decisions for Lake Wales Ridge habitats. Although most variation is found within populations, and conserving only the Carter Creek population would result in approximately 80% of the genetic variation found in my study

being retained, I also found some evidence of limited gene flow among populations. This pattern is somewhat unusual for an endangered species with a highly restricted and fragmented habitat, but is shared with several other plant species found on the ridge (Dolan et al. 1999; McDonald & Hamrick 1996). This suggests that the best management strategy for *P. myriophylla* and other high-diversity, low-structure species on the ridge is to focus on a few sites with larger populations rather than many sites with small populations. Sites where all of these plants co-occur are of particular importance for conservation, as they represent an excellent opportunity to preserve a large amount of genetic diversity for multiple species, and management at these sites should pay particular attention to the needs of these species.

Although the scrub endemic plants found on the Lake Wales Ridge share the same habitats and histories, their differing demographic characteristics result in a range of genetic patterns and very different management needs. Demographic characteristics can be broadly associated with particular genetic patterns, for instance self-fertilization with low diversity and high inbreeding, these associations cannot be relied upon to accurately predict the amount and distribution of genetic diversity in any particular species. In the case of *P. myriophylla*, despite a capacity for self-fertilization, low sexual recruitment, and highly specific habitat requirements that might have predicted low diversity within populations and extensive genetic structure, I found high genetic diversity within populations (and overall) and only moderate genetic structure. This highlights the importance of performing genetic studies for multiple species in a habitat before devising a conservation strategy for that area.

Future research

Despite the high levels of genetic diversity found in this and previous studies of *P. myriophylla*, there is still cause for concern. Loss of diversity in some populations (i.e. Dr. P and CTC A) shows that this species is sensitive to reduced population sizes, isolation, and potentially to changes in habitat or disturbance regime. Changes in genetic diversity are not immediately evident, and *P. myriophylla* relies extensively on clonal recruitment which can further delay genetic evidence of small populations. As a result, we are likely not yet seeing the genetic effects of the extensive loss of scrub habitat that has occurred in Central Florida, and especially on the Lake Wales Ridge, over the last 30 years (Turner et al. 2006; Weekley et al. 2008).

The unexpected relationship between high levels of clonal reproduction and high levels of genetic diversity points toward an important role for clonal reproduction in the maintenance of genetic diversity in *P. myriophylla*. This study cannot determine whether there is a causal link between the two measures; additional research will be necessary to address this question.

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