NOVA SCOTIA Natural Resources HABITAT CONSERVATION FUND
Contributions from Hunters and Trappers

Title: Conservation of critical lakeshore habitat in the Tusket Riverwatershed

2005 Final Report for the Nova Scotia Habitat Conservation Fund

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Executive Summary. Our research initiative exploring the reproductive biology and conservation genetics of the most endangered member of the Atlantic Coastal Plain Flora (ACPF), *Coreopsis rosea* (CORE) in Nova Scotia has been very successful. Our project goals included a) an examination of the reproductive biology of including an examination of the presence and impact of self-incompatibility in CORE in both Nova Scotia and Massachusetts b) the completion of our analyses concerning the biogeographical structure of populations of CORE across its range and c) the development of strong relationships with local landowners and conservation groups. Of the five main objectives 2005 grant application, which included studying the reproductive biology, pollinator ecology, pollinator landscape, population structure and education initiatives in CORE, we have progressed on or completed projects associated with all of them except for the pollinator landscape research because this project was compromised by flooding of the natural populations of CORE. Population size estimates in CORE reveal that the number of flowering individuals is low in all populations except for that on Wilson's lake, particularly Wilson's Lake Reserve. The results of detailed hand self and cross-pollinations in CORE revealed the presence of a genetic self-incompatibility system in both NS and MA populations. However, there is some indication that the system is breaking down and that small populations suffer from a lack of compatible pollen. Our conservation genetics work has been completed for CORE in NS and this analysis reveals that populations of CORE in NS have very low genetic diversity and are suffering from the consequences of small population genetic effects: there appears to be low levels of gene flow among populations and a significant impact of random genetic drift. Although we were unable to complete our experiment regarding the pollinator diversity and abundance across temporal and spatial scales in CORE because of flooding, we hope to continue this work in the future. Lastly, we continue to work closely with landowners in the NS populations of the suite of species in the ACPF including CORE and maintain contact with local governmental and environmental groups. Collectively, this research has shown that populations of CORE in NS are in real endanger of extinction. On-going research into the impact of cottage development on plant-pollinator interactions is needed. Finally, our interactions with the local land owners has been very positive and on-going involvement of the landowners with the preservation of this unique habitat and the ecological processes inherent to it are needed to ensure its survival.

BREEDING SYSTEM

Summary of objectives. Determine whether CORE can produce selfed seed. If CORE cannot produce selfed seed, determine how many compatible mating partners there are in a population. Determine the mean outcrossed seed set of CORE after receiving different pollen loads. This will be used to determine if and why natural populations are pollen-limited.

Breeding system Coreopsis rosea

Plants presenting sporophytic self-incompatibility (SI) usually have low or no adherence or germination of pollen to the stigmatic surface and no penetration or growth of the pollen tube into the style after a self or incompatible pollination. After an incompatible pollination, callose bodies are associated with the growth of pollen tubes. Callose fluoresces under UV light when the tissue is stained with aniline blue (0.1% KPO_4). In addition, after a self-pollination, pollen grains often detach from the stigmatic surface and are unable to germinate or grow into the style. On the other hand, after a compatible cross-pollination, none of these events are expected. If two different individuals share the same self-incompatibility mating type, it is physiologically equivalent to a self-pollination. Endangered species which present a SI system may have low fertility because it is difficult for individuals to find compatible mates via cross-fertilization (due to low population size) and the inability to self-fertilize. This becomes a more severe problem in small populations.

In the summer of 2005, we initiated the following experiments in the field. We 1) counted the size of each of eight populations (on 4 lakes) of CORE 2) performed controlled hand- self and cross pollinations to look for the presence of a self-incompatibility system 3) harvested stigmas in each population to look for a correlation between pollen deposition and population size and 4) collected fruits from self, cross and open pollinations to study the relationship between population size, number of pollen donors, and inbreeding depression.

Population size was estimated as the number of flowers over the water level and a "population" was any patch of individuals separated by at least 250m from another such patch. The number of flowering individuals per population is listed in Table 1. This covers most of the populations of CORE known in NS and therefore indicates the fairly extreme vulnerability of this species.

Table 1 Estimated population size of populations of *Coreopsis rosea* in Yarmouth County N.S. based on the number of flower heads above the water level. A population was defined as a patch of plants that grew on the shore of the lake separate from the next patch at least by 250 m of shore line. Population locations shown in Figure 2.1a.

Region	Lake	# of flower heads
Nova Scotia	Bennett	609
Nova Scotia	Bennett	294
Nova Scotia	Bennett	470
Nova Scotia	Bennett	33
Nova Scotia	Wilson	568
Nova Scotia	Wilson	2499
Nova Scotia	Sloans	209
Nova Scotia	Salmons (2005)	5
Nova Scotia	Salmons (2006)	

Determination of self-fertility in CORE material and methods. We evaluated the presence of self-incompatibility in CORE in populations from NS by performing hand self- and cross pollinations on 4 different plants from Bennett's lake, 6 plants from Wilson's Lake and 4 plants from Sloan's lake in August, and from two ponds in Massachusetts, Mary Dunn's and Cook's ponds. Hand pollinations were performed with the aid a tooth-tick. For self pollinations, pollen was collected from a different flower within the same flower head and applied to the stigma using five touch-pollinations. For cross pollinations, pollen was collected from at least two different individuals from the same population, and applied with five touch-pollinations. For plants growing in the greenhouse, flowers were collected 1, 3, 6, 9, 18 and 24 hrs after pollination. On each plant 4-6 flowers were collected for the each time-treatment combination and the pistils were analysed for pollen tube growth. To this end, pistils were first fixed in Carnoy's solution for one hour, and then transferred to 70% ethanol until further processed. Microscopic observations were performed on styles mounted on a microscopic slide with the addition of one drop of aniline blue dissolved in 0.1% KPO_4. The stain was absorbed with the aid of a filter paper after five minutes and permount was added to the edges of the coverslip. Microscopic observations were done using a Nikon E400 microscope with epi-florescent UV filter (EX: 340- 380; DM: 400; BA: 435-485). In addition, the proportion of pollen grains that adhered and germinated per flower per treatment in US and NS populations was scored (4 plants each location, 4-5 flowers/time/treatment (self or cross)).

Results. Self-incompatibility was found in both Canadian and US populations. However, there is some suggestion that the self-incompatibility response is breaking down or that the number of compatible mates in the populations, particularly in Nova Scotia, is low. The adherence and germination of self and cross pollen was similar between 1 and 6 hours after pollination (not shown). Pollen germination began ~3 hr after pollination and

reached a peak 6 hr after pollination. But differences in callose deposition and germination were observed after 9 hr: self pollen grains exhibited more callose deposition (i.e. SI reaction) and lower pollen germination after 9hr. After 18hr fewer self pollen grains remained on the stigmatic surface and some of them were found to be detached with strong callose deposition within the pollen tube (Fig 1). However, 24 hr after pollination, both self and cross pollination treatments showed lower pollen adherence. No pollen tubes were observed in the style, suggesting that pollen tube growth is slow and may commence one day after pollination. These results suggest that 1) most of the crosses within a population are incompatible or 2) the plants are showing a breakdown of the self-incompatibility system.

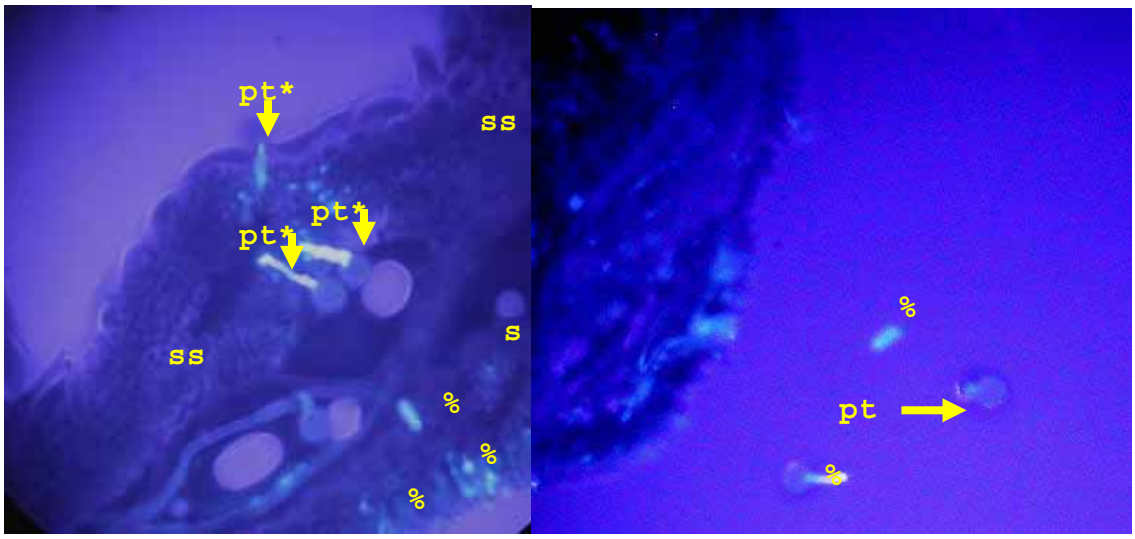

Figure 1. Microphotograph of *C. rosea* stigma stained with aniline blue (0.1% KPO_4) and visualized with epi-fluorescence (UV filter). Top. Pollen tubes growing and stopping (indicated with an arrow pt*) before penetrating the stigmatic surface (ss) 9 hr after self pollination. Population Mary Dunn, USA. Bottom. Pollen tubes growing and stopping (indicated with an arrow pt*) before penetrating the stigmatic surface (ss) 9 hr after self pollination. Note the presence of detached pollen tubes that have fallen off of the stigmatic surface after self-pollination (%) Population Mary Dunn, USA.

POLLINATION ECOLOGY

Summary of Objectives. Determine visitation rates and pollinator movement within populations of CORE to examine effect of population size and fragmentation on pollinator movements and efficiency of pollinator services. Perform controlled pollination treatments in different populations using the number of seeds per fruit and germination rates of seeds to examine effects of population size on inbreeding and reproductive fitness

Number of compatible mates in natural populations of *Coreopsis rosea*

We were unable to complete the pollinator movement research because of the shortness of the field season. A few pollinators were observed and collected, but no analyses initiated due to the briefness of the field season. However, the effect of

population size on the amount of pollen deposited in natural populations was evaluated by collecting pistils from flowers in populations of varying size and then measuring three indicators of pollination success: 1) Pollen adherence, 2) Pollen germination, an indicator of compatibility between mate pairs and 3) pollen tubes growth in the style, which is an indirect indicator of a successful fertilization.

Population size was estimated as the number of flowers over the water level and a "population" was any patch of individuals separated by at least 250m from another such patch. The experiment was only completed on two lakes because of flooding:- on Salmons lake and Sloans lake, Wilson's and Bennett's lake populations were completely submerged prior to the completion of the field season. Eight patches of variable sizes were found at Salmons and 24 at Sloans. On each one of the patches two to five flower heads were collected, preserved and prepared for microscopic imaging as described above.

Results

For each pistil, the number of pollen grains that adhered, germinated and grew into the style was recorded. There was very low overall germination and growth of the pollen tubes in all populations. The smallest patches, with 2, 4, and 5 flowers per patch, showed the highest pollen adherence, but all patches showed very low germination and growth (Figure 2). The patch with the highest germination and growth was the largest patch consisting of 76 flower heads (Figure 2). A correlation analyses of the relationship between patch size and pollen adherence, germination and growth shows that patch size was inversely correlated to the number of pollen grains that adhered to the stigma ($r=-0.334$, $p<0.001$), positively correlated to the number of pollen grains that germinated ($r=0.223$, $p<0.005$) and positive correlated to the number of pollen tubes growing in the style ($r=0.328$, $P<0.0001$). This suggests that although small patches had high pollen adhesion, larger patches show greater potential for reproductive success as a function of the breeding system. It also suggests that the presence of the self-incompatibility system is limiting opportunities for reproduction in this herb. This analysis was performed based on the 2005 data for Salmon's lake and is being examined for the 2006 data from Sloan's lake.

Unfortunately our study population was flooded twice and the number of crosses we performed was lower than anticipated and we were unable to collect the fruits. Although flooding is a natural part of the ecosystem in some years, this year populations were flooded because of work performed on a hydro-electric dam in the river system. The results from the detailed microscopic observations suggest that there is a self-incompatibility system in Nova Scotian and US populations of CORE, however the low germination rate of cross pollen suggests that there is low diversity at the S-locus in natural populations. We find lower pollen adherence of self-pollen grains than outcross pollen grains to stigmas after pollination and strong evidence of a self-incompatibility reaction which is characterised by the deposition of callose plugs at the base of the pollen tube that prevents further growth. Detailed microscopic examination of pollen tube adherence, germination and growth in another member of the family Asteraceae *Senecio squalidus* finds that the incompatibility reaction occurs after 3 hr (Hiscock et al. 2002), whereas we find that the self-incompatibility response in CORE starts after ~ 9 hr as witnessed by both the deposition of heavy callose deposition at the stigmatic surface and

the active detaching of pollen grains approximately 18 hr after pollination has occurred. We think this delayed response could be part of the breakdown of the system.

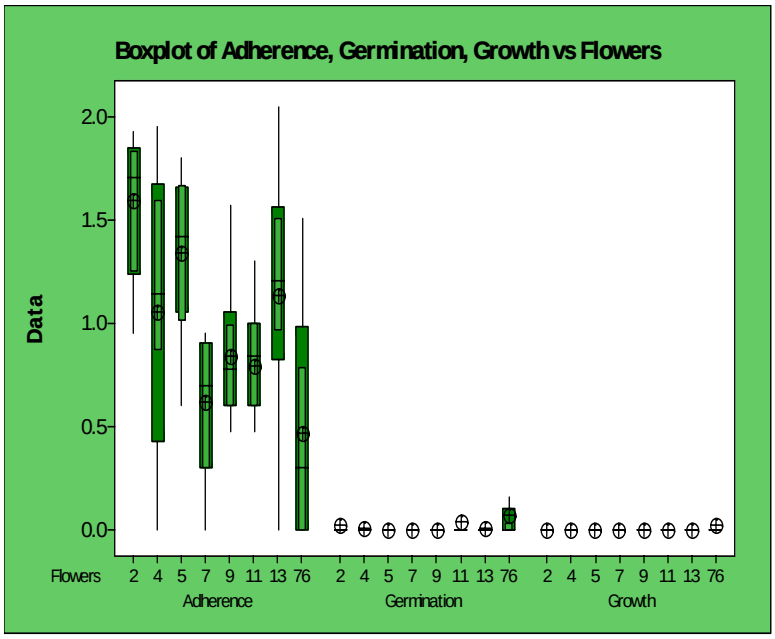

Figure 2 Mean number pollen grains that adhered, germinated and grew into styles for individuals from eight different patches of *Coreopsis rosea* growing at Salmons Lake, Canada. Numbers on the x axis refer to the population size of the patch.

The low adherence and germination of pollen, while dramatic, is a characteristic of self-incompatible members of the family Asteraceae. In CORE, we find that the average number of pollen grains per stigma is 0.75 at 18 hrs and only 1% of these pollen grains had germinated after 24 hrs. Therefore, these data strongly suggest that CORE has a self-incompatibility system, even if it is slightly weaker than in some other members of the Asteraceae. We also find evidence that the small patches of CORE may be further compromised by reduced availability of compatible pollen. This is not surprising since wild populations of CORE are small and are subject to considerable random genetic drift (see the results of the proceeding section on genetic diversity in CORE). We are in the process of evaluating whether cross-fertilization between individuals on different lakes increases the opportunities for successful fertilization. Based on the small data set we have collected here, it appears that seed production will only occur in patches that display more than 11 flowers simultaneously. This work will give us a further insight on the fecundity of the populations and the potential that they have to growth and maintain themselves as viable populations.

Summary of goals achieved

The results from the detailed microscopic observations suggest that there is a self-incompatibility system in Nova Scotian and US populations of CORE, however the low germination rate of cross pollen suggests that there is low diversity at the S-locus in natural populations. We find lower pollen adherence of self-pollen grains than outcross pollen grains to stigmas after pollination and strong evidence of the self-incompatibility

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3. POLLINATOR LANDSCAPE ECOLOGY. Summary of Objectives

a) determine the pollinator guilds present throughout the season
   i. determine if the pollinator guild present early in the season is the same guild present later in the season and pollinating CORE.

b) correlate habitat type with pollinator type throughout the season
   ii. identify those habitats that provide the appropriate pollinators for CORE, or those habitats which are detrimental to CORE receiving pollinators, ie. by providing nectar or pollen competition from other flowers.

This part of the project could also not be completed due to flooding. Dishes were placed in the field when the water levels went down during the summer. The water level went down sufficiently to expose the shoreline in mid July (much later than usual). Dishes were placed on the shoreline for 24 hours every 10 dyas from mid-July to late August and several insects collected. However, the plan was to sweep for insects on Coreopsis during the field season and to increase the number of pan traps during the flowering season of CORE. This was not possible because the water levels began to raise again in late August.

4. POPULATION CONNECTIVITY

Summary of Objectives. Determine levels of genetic variation within and between populations using nuclear and chloroplast markers and use this information to determine the mean levels of gene flow among populations, the main pathways of seed dispersal,

and to determine whether populations suffer from inbreeding or small population genetic effects.

Background

Population size is the most important criterion to consider when evaluating the extinction threat of a species (Frankham et al., 2002). Small population sizes typically arise in plant species as a result of habitat fragmentation and destruction (Ellstrand and Elam, 1993). Environmental change may result in a population bottleneck where there is a dramatic decrease in population size and only a few individuals survive to form the next generation. A population bottleneck typically coincides with a loss of genetic diversity (especially rare alleles) because the surviving individuals are not representative of the genetic variation originally present in the population. A founder event, where a small number of individuals migrate to establish a population in a new location, has similar genetic consequences to a population bottleneck (Barrett and Kohn 1991). A decrease in population size will produce a decrease in genetic diversity, just by chance. Thus endangered or rare species experiencing small population sizes tend to have less genetic variation than non-endangered species Frankham (1995). Frankham (1995) collected allozyme data for 38 endangered species and their non-endangered relatives. As expected, 32 out of the 38 endangered species studied had lower allozyme diversity than their non-endangered counterparts (Frankham, 1995).

If a population remains small after experiencing a bottleneck or a founder event, genetic drift will perpetuate the loss of genetic diversity within the population (Barrett and Kohn, 1991). Random genetic drift causes small populations to experience a random and unpredictable departure from their original allele frequencies, just by chance. In small populations undergoing genetic drift there is an increased risk that alleles will go to fixation or be lost entirely from a population simply because the parental generation can only pass on a subset of their alleles to their offspring (Ellstrand and Elam, 1993). The random fixation or loss of alleles within a population also serves to decrease genetic variation within a population (Frankham et al., 2002). Gene flow opposes the effects of genetic drift by homogenizing allele frequencies across populations (Ellstrand and Elam, 1993). Unfortunately, endangered plant populations are often separated by distances greater than their maximum dispersal distance (Barrett and Kohn, 1991). “Isolation by distance” refers to the theory that populations will differentiate and experience local changes in allele frequencies when geography limits dispersal between them. This theory implies that genetic distance and genetic differentiation increase with geographic distance (Slatkin, 1993).

Here we address the population genetic structure of the Atlantic Coastal Plains Flora species *Coreopsis rosea* (CORE). The Atlantic Coastal Plain Flora (ACPF) is a group of 64 wetland plant species, 11 of which are protected by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). These plants inhabit the Atlantic Coastal Plain: a low, flat region with abundant freshwater that extends along the eastern coast of the United States. Atlantic Coastal Plain habitat is also found near the Great Lakes and along the shorelines of lakes and ponds in southwest Nova Scotia (Elderkin et al., 2004). The Atlantic Coastal Plain was formed during the last glaciation and it is characterized by glacial tills of sand and gravel and low nutrient, gradually sloping shorelines with fluctuating water levels (Keddy, 1985).

It is somewhat of a mystery how the warm-adapted Atlantic Coastal Plain Flora came to establish disjunct populations as far north as Nova Scotia. At the end of the Pleistocene glaciation sea levels were lower than at present resulting in the exposure of parts of the continental shelf. Approximately 11 000 years before present regions of the continental shelf known as Browns Bank and Georges Bank were exposed in between southwest Nova Scotia and Cape Cod. These exposed offshore banks could have facilitated migration of species from Massachusetts to Nova Scotia (Shaw et al., 2002). Green (1986) found evidence for the migration of tree species along this "land bridge", suggesting a pathway by which the ACPF may have arrived in Nova Scotia. There are two hypotheses regarding the expansion of the ACPF into Nova Scotia approximately 11 000 years ago. One hypothesis suggests that the ACPF refuged exclusively in South Carolina during the last glaciation and then expanded north with the warming of the climate. On the other hand, refugia of ACPF may have also existed on the exposed continental shelf somewhere near Nova Scotia in addition to the refugia in South Carolina.

Methods

Sampling of *Coreopsis rosea*

Leaf material was obtained from 4 lakes in Nova Scotia and 3 ponds in Massachusetts for CORE (Table 2, Figure 3). Leaf material was kept on ice during sampling and then frozen at -80°C. DNA was promptly extracted from the plant tissue using the CTAB protocol (Doyle, 1991) or a Qiagen™ DNeasy Plant Mini Kit. The DNA samples were run out at 100V on a 0.8% agarose gel along with Amersham Biosciences™ Lambda DNA-Hind III Digest as a DNA marker. The gel was visualized under UV light and the DNA samples were quantified.

Table 2. Coordinates (latitude and longitude) of populations of *Coreopsis rosea* (CORE) in southwest Nova Scotia (NS) and Massachusetts (MA) (Google Earth, 2006).

Population	Species	Coordinates
Bennetts, NS	CORE,	43°55'42.15"N 65°54'10.21"W
Wilsons, NS	CORE	43°56'23.16"N 65°53'31.69"W
Sloan, NS	CORE	43°58'44.09"N 65°55'45.66"W
Salmon, NS	CORE	43°51'43.71"N 66°00'52.69"W
Mary Dunn Pond, MA	CORE	41°40'30.39"N 70°16'44.63"W
Cooks Pond, MA	CORE	41°55'20.40"N 70°39'54.75"W
Harlow Pond, MA	CORE	41°55'11.94"N 70°40'12.58"W

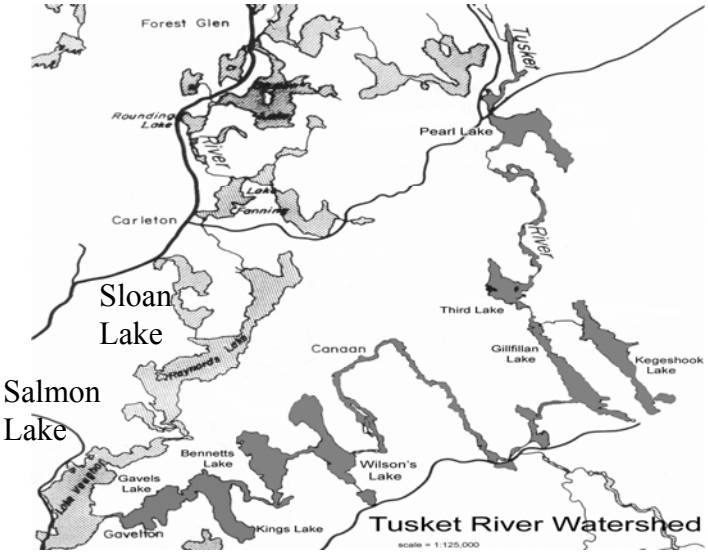

Figure 3 a Map of the distribution of studied Nova Scotian populations of CORE.

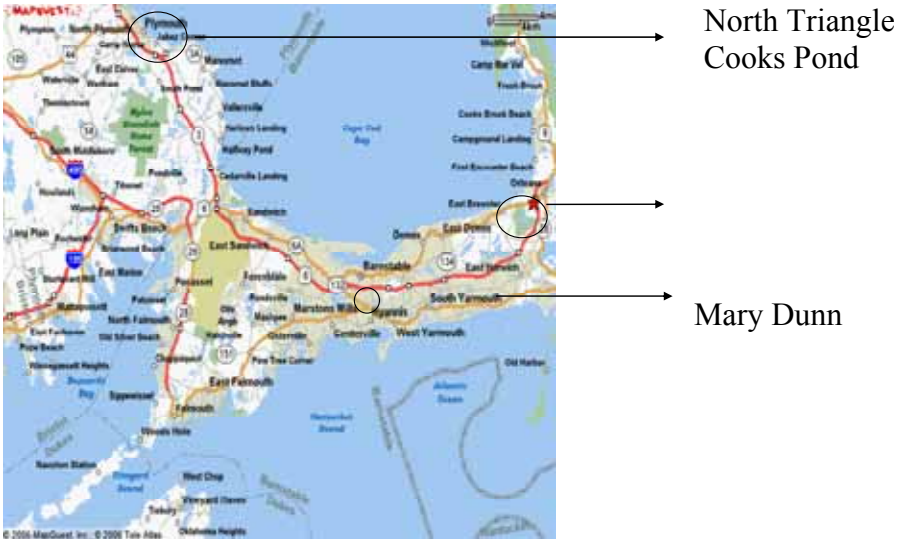

Figure 3 b Map of the distribution of populations of CORE sampled from Massachusetts

ISSR marker and Chloroplast DNA sequence data collection for CORE and SAKE

The DNA samples were subjected to amplification via the polymerase chain reaction (PCR) technique for nuclear and chloroplast markers. For the chloroplast markers, DNA samples of CORE were amplified for a rapidly evolving intergenic spacer in the chloroplast genome between the genes trn H and psb A (Hamilton, 1999). For the nuclear genetic analyses, the samples were PCR amplified using primers complementary to a microsatellite repeat to generate Inter Simple Sequence Repeats (priers 840 and 841, Culley) which amplify rapidly evolving regions of the nuclear genome and provide a "fingerprint" of band absence and presence for each individual, i.e. they are a dominant marker.

PCR's were carried out as described in Wood (2006). For the chloroplast marker, PCR products were sequenced by the lab technician using an MJ Base Station. Sequence

data was edited and the consensus sequences generated using SeqManII (DNASTAR Inc.). A multiple sequence alignment was obtained using ClustalW (MEGA 3.1). For the ISSR data, PCR products were run out on polyacrylamide gels using an Amersham Biosciences™ CleanGel DNA Analysis Kit Norgen™ and visualized with a silver stain as outlined by Caetano-Anollés and Gresshoff (1994). Digital images were obtained of the CleanGels and the gels were scored using ImageQuant TL Image Analysis Software (Amersham Biosciences™). A matrix was generated for each gel with each individual represented by a row and each locus represented by a column. Individuals received a score of '1'if a band presence was observed at a locus and a score of '0' if a band absence was observed at a locus.

Analyses of ISSR data: The ISSR data were primarily analyzed using the publicly available software *Tools for Population Genetic Analyses* (TFPGA) (Miller, 1997). A band presence was assumed to represent a dominant genotype while a band absence was assumed to represent a homozygous recessive genotype. Allele frequencies were estimated using the method presented in Lynch and Milligan (1994). Using the estimated allele frequencies, unbiased expected heterozygosity (Nei, 1978), gene diversity and the percentage of polymorphic loci using a 95% criterion were calculated. In addition, the values of F_{st} were calculated to examine the amount of genetic differentiation observed among populations (and not within populations) relative to the total genetic variation using the Weir and Cockerham method (1984) as $\hat{\theta} = \frac{a}{(a+b+c)}$ where a = the variance between populations, b= the between variance between individuals within a population, and c= the variance between gametes within an individual. (F_{st}) was calculated among populations and among subpopulations. S represented the differentiation of subpopulations relative to total diversity while P represented the differentiation of populations relative to total diversity. The effective number of migrants per generation ($N_e m$) to/from each subpopulation or population was calculated from the (F_{st}) value using the relationship: $F_{ST} = \frac{1}{1+4N_e m}$ (Wright, 1931).

A Principal Components Analysis was performed using the data for CORE in Minitab 14 (Minitab Inc.). The matrix of 0's and 1's generated for ISSR primer 840 was compressed into two axes: PCA1 and PCA2. Each individual was assigned a pair of coordinates based on its multi-locus fingerprint and then the individuals were plotted on the PCA1 and PCA2 axes. Nei's unbiased estimates of genetic distance and genetic identity (1978) were computed using the allele frequencies obtained by the method of Lynch and Milligan (1994). A comparison of the genetic to geographic distance between populations was made for the Nova Scotian populations by calculating the average geographic distances between populations in Nova Scotia using Google Earth (2006). The Mantel Test (Mantel, 1967) was performed to determine if there was a significant relationship between the genetic and geographic distance matrices. Phylogenetic trees based on the ISSR data were calculated based on the neighbor-joining algorithm using the program TREECON (Van de Peer and De Wachter, 1994) and Nei and Li (1979) genetic distances between individuals. Bootstrap values at the nodes were obtained by performing 1000 replications of sampling a subset of the data with replacement and generating a tree from that subset. The proportion of times that the grouping shown in the

final tree was obtained during bootstrapping is indicated by the bootstrap value (Krane and Raymer, 2003).

Analyses of Chloroplast DNA sequence data. Because CORE exhibited very low levels of DNA sequence polymorphism, limited data analyses could be performed. Phylogenetic trees based on the chloroplast sequence data were constructed for CORE by analyzing the 350 bp sequence data using jukes-cantor correction for multiple hits and neighbour joining algorithm.

Results

Coreopsis rosea

Chloroplast sequence data:

Extremely low levels of DNA polymorphism were observed in CORE for the chloroplast DNA sequence. Only four of the 41 individuals sequenced exhibited any nucleotide changes. Two of these individuals were from Bennetts lake and two from Massachusetts (not shown).

ISSR data The ISSR data demonstrated low expected heterozygosity values in all populations (Table 3). A positive relationship was observed between expected heterozygosity and population size (Figure 3). Wilsons Lake had the largest population size and the largest expected heterozygosity while Salmon Lake had the smallest population size and the smallest expected heterozygosity (Table 3).

Table 3. Genetic variation at 18 ISSR loci in five populations of *Coreopsis rosea*. Population size (*N*), sample size per locus (*n*), number of polymorphic loci (*P*), percentage of polymorphic loci (%*P*), and unbiased expected heterozygosity averaged over all loci (*h*) are shown. Mean values for percentage of polymorphic loci and expected heterozygosity were also calculated.

Site	*N*	*N*	*P*	*%P*	*h*
Bennetts	1406	8	1	5.556	0.0296
Wilsons	3067	13	5	27.78	0.1369
Sloan	209	6	1	5.556	0.0320
Salmon	120	4	0	0.0000	0.0000
Mary Dunn	1000	8	2	11.11	0.0458
Mean	1160.4	7.8	1.8	10.00	0.0489

The F-statistics show that the overall levels of differentiation of subpopulations in NS is high (S =0.35) (Table 4). Including both NS and MA populations, indicates that differentiation among subpopulations is lower (S =0.19) and differentiation between the US and Canada even lower (P = 0.29). This suggests that random genetic drift has caused greater divergence in allele frequencies between populations than suggested by the divergence of allele frequencies between regions. The estimated effective number of migrants per generation is estimated to be 0.1967 migrants per generation between all subpopulations, 0.45 within NS only and 0.5980 migrants per generation between NS and MA (Table 4).

Table 4. F-statistics over all loci based on ISSR primer 840 data and effective number of migrants per generation (N_em) for *C. rosea* populations.

Populations	F_{st}	F_{st} value	N_em
NS and Mass.	*S*	0.5596	0.1967
NS and Mass	*P*	0.2948	0.5980
NS	*S*	0.3545	0.4552

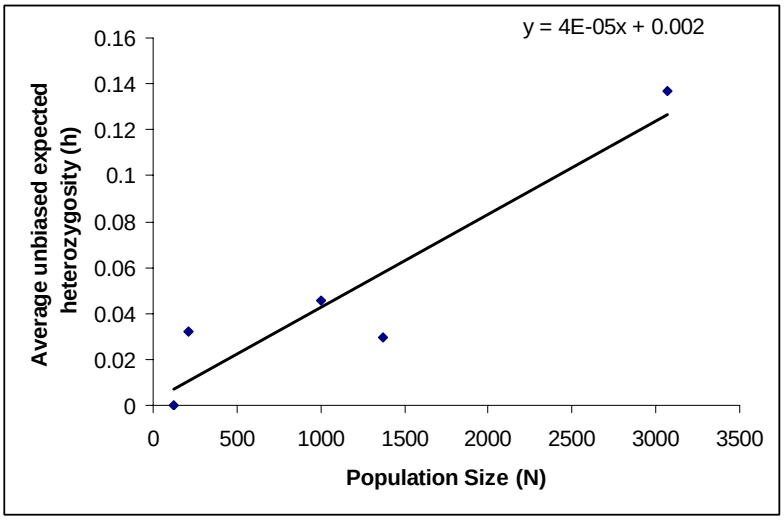

Figure 4. Average unbiased expected heterozygosity (*h*) versus population size (*N*) for the *C. rosea* populations in MA and NS populations

The Principal Components Analysis demonstrated that the Mary Dunn Pond individuals had multi-locus fingerprints which were unique from those in Nova Scotia (Figure 5), Wilsons Lake formed a loose cluster and Bennets and Wilson's were closely related.

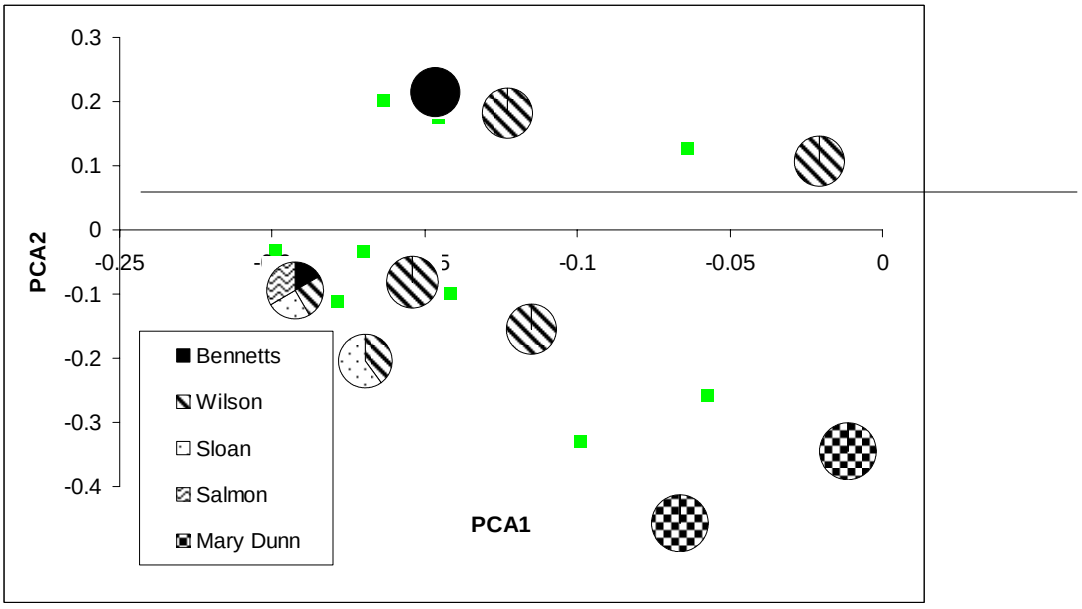

Figure 5. Principal Components Analysis performed using ISSR primer 840 data.

The genetic distances between the Nova Scotia subpopulations were very low ranging from 0- 0.0773 using the primer 840 data. The Mantel Test showed no relationship between genetic and geographic distance among Nova Scotia CORE subpopulations. The correlation between geographic and genetic distance was essentially 0 and the two tailed Z-test did not find a significant difference between the original Z-score and the Z-score based on the null hypothesis.

A neighbor-joining tree derived from the primer 840 data grouped the Mary Dunn Pond individuals apart from the Nova Scotia individuals (Figure 6). Most of the Wilsons Reserve individuals grouped together and they appeared to be the most closely related to the Mary Dunn Pond individuals. Most of the Bennetts individuals formed a separate clade. The Salmon individuals grouped together in the same clade along with representatives from the individuals while the other half of the Sloan individuals grouped with the Salmon her three s.

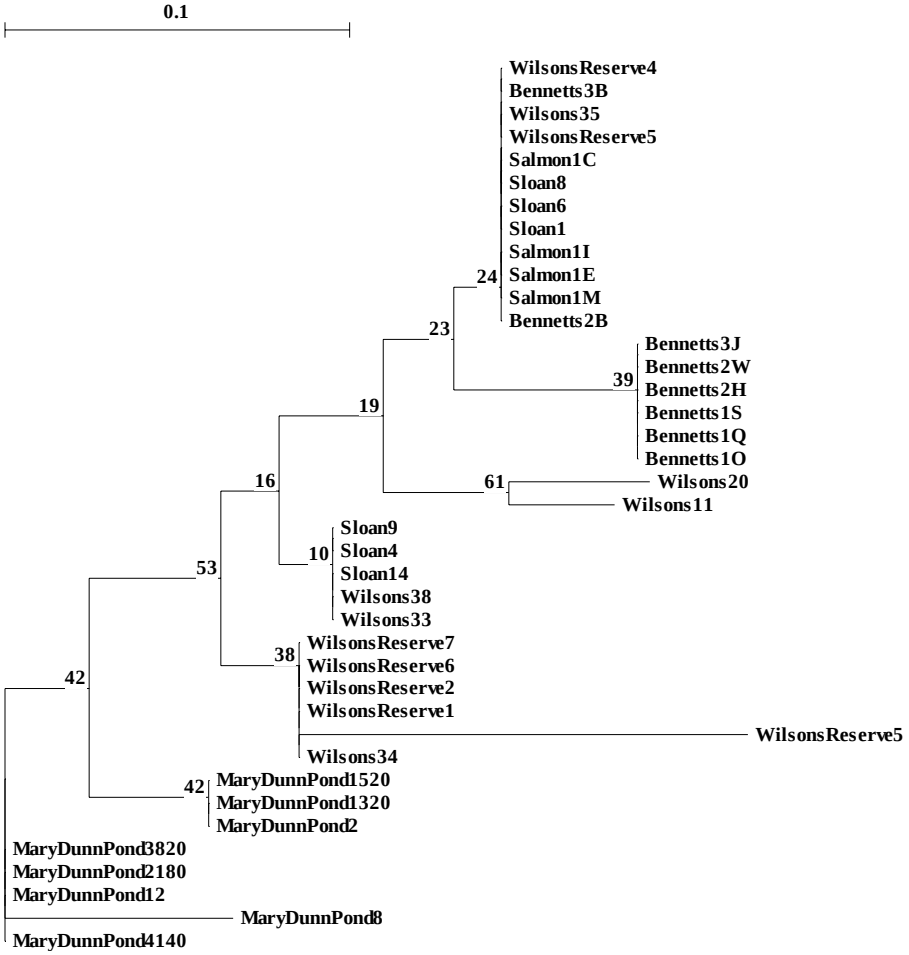

Figure 6. Neighbor-joining tree constructed using ISSR primer 840 data for 39 *C. rosea* individuals from Massachusetts and Nova Scotia. Bootstrap values were calculated as percentages of 1000 replications. The tree was rooted using the Mary Dunn Pond sample 4140.

Summary

Coreopsis rosea

Populations of CORE in Massachusetts and Nova Scotia suffer from the characteristic low heterozygosity and polymorphism expected in endangered species. Both US and Canadian populations of the species show low to no variation at the chloroplast marker we used (which is one of the fastest evolving region of the chloroplast genome) however, the United States populations of CORE harbour more genetic variation than the Nova Scotia populations at the nuclear marker. Although human fragmentation of CORE populations threatens this species, recent disturbance cannot be held entirely responsible for the endangered status of CORE. The proposed historic population bottleneck during the Pleistocene is probably responsible for the lack of genetic variation in CORE populations since other members of the Atlantic Coastal Plains Flora in NS and MA show low to no overall diversity (*Drosera filimormis*, Cody, 2003; *Polygonella articulate*, Lewis and Crawford, 1995, and *Sabatia kennedyana*, Good-Avila, WWF report, 2006). CORE is hypothesized to have resided on the continental shelf near Nova Scotia during the last glaciation and subsequently spread to Nova Scotia and Massachusetts approximately 11 000 years ago. ISSR and chloroplast sequence data from South Carolina populations of CORE are necessary to further investigate this hypothesis. Despite low levels of genetic diversity, population differentiation was observed as a result of genetic drift. Small population size, restricted migration, and clonal reproduction combine to make genetic drift a strong evolutionary force in CORE populations. Isolation by distance was observed between the Nova Scotia and the Massachusetts populations of CORE; however, genetic drift appears to have erased any evidence of isolation by distance in Nova Scotia. Given the low levels of heterozygosity and polymorphism in CORE the species is probably more vulnerable to disease outbreaks (Peakall et al., 2003), less able to reproduce sexually, and less able to cope with environmental change such as cottage development and all-terrain vehicle disturbance (Frankham, 2003; Ellstrand and Elam, 1993). In short, CORE is in extreme danger of extinction in Nova Scotia.

5. EDUCATION Summary of objectives

Work with local landowners and public tourist on understanding and stewardship of ACPF species. Present findings of study at local conservation group meeting (TREPA). Continue to present results to Recovery Team. Present findings at national/international conference

Summary of activities: Similar to ecological processes, conservation initiatives and dissemination of acquired knowledge can be addressed at multiple scales. Our initiative focussing on the conservation of Atlantic Coastal Plain Flora (ACPF) is intimately associated with land owners, local conservation groups, national recovery teams, and conservation ecologists internationally (listed here by increasing scale). We were fortunate enough to work directly with 3 landowners (as each of our disturbed sites was situated next to a cottage) in 2004 and in 2005 we worked with an additional 4 landowners regarding access to populations of CORE. In each case, we formed excellent working and personal relationship with these land stewards. We were also in touch with

the Tusket River Environmental Protection Association (TREPA), keeping them up to date on our research and findings. We have prepared a general summary of the results of the pollination ecology and seed bank work, part of the masters thesis of Andrew Trant, that we are considering publishing in a local newspaper and/or printing for the landowners: we will forward this to your office when complete. Furthermore, we have attended meetings with the ACPF Recovery Team to get feedback on our study and also to share our results and knowledge with them. On multiple occasions this summer, we worked directly with members of the ACPF Recovery Team and the Department of Natural Resources to enhance shoreline protection and kept them informed on destructive activities that we witnessed first hand. In the summer of 2005 Andrew Trant travelled to Brasil to present a paper at a conference of the Society for Conservation Biology on the pollination ecology of SAKE. We have submitted one manuscript from this work, and are working on two more manuscripts. Disseminating acquired knowledge at all of these scales in essential for raising local awareness about the habitat needs of ACPF and for contributing to the scientific literature on the responses of different species/communities to habitat fragmentation and rarity.

Currently: We are currently focused on completely some manuscripts and getting our general summary of the work in SAKE to a local audience via newspaper or direct delivery.

Goals achieved: We were very successful at creating and maintaining meaningful relationships at the individual, local, provincial, and national level. Such relationships are paramount to the successful recovery of any species at risk.

Communicating results of project

As stated in the previous section, the findings of this study are presented to the recovery team at quarterly meetings. Part of this research initiative was recently submitted as part of the thesis requirements for MSc student, Andrew Trant and manuscripts are either submitted (1) or in the process of submission for his work. In addition, undergraduate honours student, Sarah Wood, working in my laboratory submitted her thesis on the conservation genetics of CORE in the April of 2006 and this data will be combined with more data collected in 2006 prior to submission for publication. Thirdly, a new master's student (commencing May 2005) working in my laboratory, Jolene Sutton is working on the life history and reproduction of SAKE in NS, MA and SC and the results from her work will be presented at a regional meeting this year and later in a series of talks/manuscripts. Fourthly, a post-doctoral research in my laboratory, Dr. Miriam Ferrer, is working on the reproductive biology of CORE. Her addition to the group has been critical because it is not easy to do reproductive biology in the Asteraceae and her Ph.D. involved studying the reproductive biology of a shrub in the Asteraceae. Dr. Ferrer presented the results of our research on the SI system in CORE at a conference of the regional network – the Atlantic Center for Global and Climate Environmental Research (ACGCER) – in September of 2005. At this same conference, I presented the results of our research on SAKE. The contribution of the NS Habitat Conservation Fund has been thankfully acknowledged in all oral and written communication.

Overall success of project

The overall success of a project can be quantified using various indices. Whether through

meaningful research collected, completion of pre-arranged goals, relationships formed with land stewards or knowledge shared with interested organizations – this project was overwhelmingly successful! The initial feedback that we have received has been very positive and Dr. Sherman Boates, co-chair of the ACPF recovery team is interested in our results concerning the affect of cottage development on pollinator movements. We will perform more sampling in 2006, with the aid of entomologist Steve Javorek, to determine if abundance and diversity of pollinators differs throughout the watershed. We are also very pleased with our results from the conservation genetics of populations of SAKE in NS. We anticipate that these data will shed light on the important processes by which the unique suite of ACPF in NS became isolated from US populations and the processes maintaining population diversity and connectivity within NS. These data are also of considerable interest to managers of the wild populations of SAKE in MA. Thirdly, our work on the conservation genetics of CORE are also progressing nicely and we will continue to work on the reproductive biology of CORE in an effort to understand why NS populations of CORE have low recruitment of sexually derived offspring. We feel these data could be critical for the successful recovery of this species. At the present point, it appears that flooding of populations, from both natural but also work on the hydroelectric dams in the Tusket River watershed is a major cause of low recruitment for CORE. Three of the four years we have worked in Yarmouth, CORE populations have flooded during flowering. Secondly, we have evidence that the species is pollen limited and the results of experiments in SAKE suggest that cottage development of the lakeshores may limit habitat for insects. This needs to be explored in further work. Given that our experience in the watershed also shows that cottage owners are willing stewards of the land, our research suggests that understanding the impact of cottage development on plant and animal communities and educating private land owners about these ecosystem processes are both essential for conservation. Finally, our work in the Tusket River watershed suggests that greater dialogue between conservationist and the hydroelectric dam companies is needed. We look forward to building on our results and to communicating these ideas to the general public and the scientific community.

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