Conservation of Atlantic Coastal Plain Flora Species at Risk and important lakeshore habitat in the Tusket River Watershed

2006 Final Report for Nova Scotia Habitat Conservation Fund

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Executive Summary. Our research initiative exploring the reproductive biology and conservation genetics of two high priority Atlantic Coastal Plain Flora (ACPF) has been very successful. Our project goals included a) an examination of the reproductive biology of *Sabatia kennedyana* (SAKE) across its range in North America b) an examination of the presence and impact of self-incompatibility in the endangered species *Coreopsis rosea* (CORE) in Nova Scotia and Massachusetts c) the completion of our analyses concerning the biogeographical structure of populations of SAKE and CORE and d) the development of strong relationships with local landowners and conservation groups. Of the objectives listed in our 2006 grant application, we have completed the data collection and/or analyses of all but two of our goals – we were not able to complete the section on landscape ecology (objective 6) and our inbreeding depression was not successful, but the remaining sections of our experiments have all been completed and revealed many interesting insights into the conservation of these species. In the summer of 2006, we completed our visits to North American populations of SAKE: we mapped, studied and sampled (for genetic analyses) from most of the known populations of SAKE in North America (although only the portion conducted in Nova Scotia was supported by the NS Habitat Conservation Fund). This work included studying populations on eight lakes in NS, several kettle ponds in Massachusetts (MA) and one main study site in North Carolina (NC), USA. This has revealed longitudinal variation in effort invested in clonal versus sexual reproduction across the range of SAKE and an apparent shift in pollinators: populations in NC have much larger floral displays than those in NS and appear to be pollinated predominantly by bumblebees, whereas populations in NS present many fewer flowers/rosette cluster and are predominantly pollinated by syrphid flies (Trant et al., submitted). Furhtermore a detailed examination of morphological and genetic differences in disturbed verus non-disturbed sites of SAKE in NS has revealed that there is no difference in morphological but that there are differences in genetic diversity among populations – with disturbed populations showing an increase in genetic variation and reduced spatial genetic structure compared to non-disturbed populations. For our research on CORE, the results of detailed hand self and cross-pollinations has 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 seed set is limited by a paucity of compatible pollen. Our conservation genetics work has progressed tremendously in the last year: we now have completed our data set on the genetic variation within and between populations of SAKE across its range in North America and have performed a detailed study of the genetic variation within and between populations of CORE in NS and, to a lesser extent, in MA, USA. Lastly, we continue to work closely with landowners in the NS populations of SAKE and CORE and maintain contact with local governmental and environmental groups.

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Overview of research program:

Our research initiative focuses on the conservation of the Atlantic Coastal Plain Flora (ACPF) in southwest Nova Scotia. This floral complex is comprised of over 60 mostly wetland species – eleven of which are designated as at risk by COSEWIC, with two of these species listed as Endangered and 9 listed as Threatened or of Special Concern under SARA. Many of these ACPF species occupy specific lakeshore habitat that provides a high disturbance regime necessary for their persistence. In recent years, this habitat has been subject to a 45% increase in cottage development and shoreline modification. The ACPF Recovery Team has identified changes in land-use patterns as being the greatest threat to the persistence of these species at risk.

Over the last three years we have conducted research on the conservation genetics, pollination, seed bank and reproductive ecology of the Plymouth Gentian *Sabatia kennedyana* (SAKE), a species listed as Threatened under SARA and globally rare (rank G3/S1) and Pink Tickseed, *Coreopsis rosea* (CORE), which is listed as Endangered under SARA and is also globally rare (rank G3/S1). This has revealed that pollinator movements are adversely affected by cottage disturbance (Trant et al., submitted), that the number of exotic species is greater in disturbed habitats in Nova Scotia (Trant, 2005) and that the main source of connectivity of NS populations of SAKE is probably by seed and ramet transportation via water (Good-Avila et al. in prep). Over the last year, we have expanded our research initiative to include comparative work of the life history, pollinator ecology and genetic uniqueness of NS compared to US populations of both SAKE and CORE. This has involved comparative work between populations of both species in NS, Massachusetts, and North Carolina where the species are also of special concern: SAKE is a species of special concern in MA and is imperiled in the Carolinas. CORE is vulnerable in MA, and critically imperiled in South Carolina (G3/S1). Lastly, we continue to work closely with land owners of privately owned cottages in NS – an initiative that has been very productive for stewardship. An understanding of the biology and uniqueness of Canadian populations of SAKE and CORE is urgently needed for conservation.

Section I: Reproductive Biology of Sabatia kennedyana

Summary of Objective as in grant

a. Determine life history characters, such as amount of asexual vs sexual reproduction, flower number, stalk height etc. in four populations of SAKE, two from NS, one from MA and one from NC with a focus on comparing disturbed vs undisturbed habitat. We are only requesting research funds for the NS portion of this work from the NS Habitat Conservation Fund.
b. Determine if asexual reproduction increases (and if genetic diversity consequently decreases) in fragmented populations.
c. 1) map the location of a series of focal maternal plants 2) obtain demographic information about density or levels of fragmentation in the sampled maternal plants 3) collect 10-20 progeny/maternal plant and score them for paternity. This will allow us to estimate the impact of habitat fragmentation on pollen dispersal in SAKE.

Background.

In plants, mating patterns may be strongly influenced by the size and structure of populations and the availability of resources such as light, temperature and nutrients. Regional variation in key population characteristics may result in selection for different evolutionary pressures regarding reproductive and life history traits across the geographic range. Although hotly debated, many authors adhere to a model in which species distributions are thought to conform to the ‘‘abundant center’’ model, in which population size, number and density are highest at the geographical center of a species’ range and decline towards the periphery (Sagarin and Gaines, 2002). However, very few studies have quantified the consequences of geographic variation in population size, density, and isolation for pollination ecology and mating. In small, isolated or northerly populations, opportunities for cross-pollination may be limited resulting in an increase in self-fertilization or asexual reproduction in these populations if the species is capable of clonal and/or self-pollination (Herlihy and Eckert, 2005). If populations differ in their degree of self-versus cross-pollination or sexual versus asexual growth they may become further isolated due to genetic and/or reproductive factors that can drive either speciation or extinction. As these pressures accrue in populations, morphological characteristics such as rosette growth and/or flower number, may diverge between populations thereby indicating how changes in morphology is correlated to changes in reproduction and breeding systems (Parsons and Hermanutz 2006). Environment factors that vary with latitude, including temperature, irradiance and photoperiod, all influence plant morphology growth and reproduction, so it is not unusual for a species to show phenotypic patterns across a latitudinal range (Pilon et al. 2002). Based on their literature review Pilon et al. 2002 suggested that phenotypic in the field may be partially genetically based, and ecotypes from higher latitudes were generally smaller and produced more seeds.

Anthropogenic disturbances have also been shown to affect phenology and life history traits. For example, anthropogenically disturbed sites, populations of both *Braya longii* and *B. fernaldii* are smaller and more patchily distributed, and individuals are larger and produce more seeds compared to naturally disturbed sites (Parsons and Hermanutz 2006). In this study, we assess the investment in clonal versus sexual reproduction across the range of SAKE. We additionally analysed the impact of disturbance on asexual versus sexual reproduction in Nova Scotia by looking at differences in morphology, genetic variation and spatial genetic structure in 6 populations, 3 disturbed and 3 non-disturbed.

Methods.

Study organism and range

Sabatia kennedyana (SAKE) is a semi-aquatic, herbaceous, lakeshore perennial that is designated as Threatened in Canada under the Species at Risk Act (SARA) and Endangered under the Nova Scotia Endangered Species Act due to declines in the species' geographic range (Environment Canada 2005; COSEWIC, 1983). This species is also of international concern, with an International Global Rank (G-Rank) of G3 and an International Sub-National Rank (S-Rank) of S1 in Canada, implying that it is rare, and vulnerable to extinction. SAKE's main range is along the east coast of the United States, including Massachusetts, Rhode Island, North Carolina and South Carolina (Environment Canada, 2005) where State designations are Special Concern, Endangered, and Threatened for the upper three states respectively. In South Carolina, this species has an International Sub-National Rank of S3.

For Latitudinal comparisons, three study locations were chosen to represent the northern tip, mid- and core of the species range: Nova Scotia, Massachusetts, and North Carolina. These three regions indicate the primary areas occupied by the species as can be seen from the map of the range and vulnerability of SAKE across its entire distribution. All Canadian populations occur disjunctly on the shores of approximately nine lakes in southwest Nova Scotia (Yarmouth County). Although Nova Scotia populations occur at the geographic northern limit for this species, this province is believed to have the second largest concentration of this taxon, next to the state of Massachusetts (COSEWIC 1983). Furthermore, due to the species' rarity in several sites along the eastern seaboard, Nova Scotia populations also represent a substantial proportion of the world population (COSEWIC 1983; 1998), suggesting that this area should be an important target for conservation and management.

Sabatia kennedyana reproduces sexually through seed production, and also exhibits extensive asexual reproduction through vegetative (asexual/clonal) growth via stoloniferous runners. Our observations suggest that once a seed germinates, it grows into a basal rosette, which develops stoloniferous runners that produce additional "daughter" rosettes. These daughter rosettes continue to be reproduced via vegetative growth, to form a spreading mat of rosettes, or a "clonal cluster".

Field work: selection and mapping of populations and traits measured.

In total, nine intensive sampling sites were established (seven in Nova Scotia (one in 2005 and six in 2006), one in Massachusetts (2005), and one in North Carolina (2006) to compare morphologies, clonality, and reproductive output across the latitudinal range of *SAKE* and within disturbed versus undisturbed habitat (Nova Scotia only). For disturbance comparisons, it was necessary to have intensive sites in disturbed as well as undisturbed habitats. Undisturbed sites were defined as being greater than 300 meters from cottages, and any kind of shoreline modification or development such as a wharf. These sites were sometimes subjected to foot traffic, and more rarely to ATV traffic, although the overall level of disturbance may be relatively low compared to disturbed sites due to differences in cottage proximity and accessibility.

Field work was conducted between 20 July and 20 October 2005, and between 5 July and 14 October, 2006. At each intensive sampling site, an area of ~500m^2 was selected for sampling. Except for the main site in North Carolina, each intensive site was established

along the shoreline of a pond or lake and was 100m long and approximately 4-8m wide (the width the shoreline, which fluctuated, but which was on average ~5m). The main site in North Carolina was 30m long and approximately 15-20m wide, and was located in a roadside floodplain between two bridges. The location of each sampled plant was determined using an X-Y coordinate system spanning the transect. Characters indicative of asexual and sexual growth were measured: stem height, number of flowers per stem, number of flower stems, flowers and rosettes per cluster, number of rosettes per flower stem, cluster diameter, fruit set, seed set. Flower stem height, the number of flowers per stem, fruit set, and seed set were recorded to compare the sexual reproductive output. All other traits were recorded to compare relative vegetative (possible clonal) output and to determine if there were differences in the effort put into vegetative versus flowering growth. Additionally, we analysed the amount of genetic variation at ISSR loci in disturbed and non-disturbed populations in both parental (leaf) and seed banks in 6 NS populations to see if standing levels of genetic diversity have been negatively, or positively, influenced by disturbance. To determine if disturbance has affected the clonal structure of populations, the change in genetic relatedness of individuals as a function of their spatial structure (using the mapped position of each clone) was further assessed.

Results.

Flower stem height and flower number: When all data from all years were pooled for each location (latitude), the mean flower stem heights (cm) for Nova Scotia, Massachusetts and North Carolina were 23.3 ± 5.05cm, 53.3 ± 11.57cm, and 80.56 ± 18.57cm respectively. The mean numbers of flowers per stem were 1.18 ± 0.47, 2.8 ± 1.98, and 10.41 ± 8.00 (Table 1.1). An ANOVA of log transformed data indicated that flower stems became significantly taller, and produced more flowers per stem as latitude decreased ($F_{2,1608}$=3505, P<2.2e-16, Multiple R-Squared=0.8134; $F_{2,1642}$=2318, P<2.2e-16, Multiple R-Squared=0.7384 respectively).

The increase in sexual reproduction with decreasing latitude is illustrated by the graph shown below of the relationship between stem height and flower number (Figure 1.1)

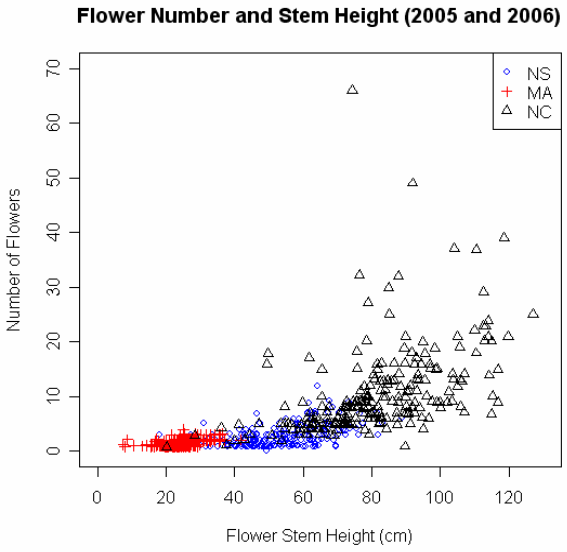

Figure 1.1 Relationship between flower stem height and the number of flowers per stem for populations of SAKE across its range in North America.

Number of flower stems per cluster and mean number of flowers per cluster: When all data from all years were pooled for each location (latitude), the mean number of flower stems per cluster for Nova Scotia, Massachusetts and North Carolina were 1.15 ± 0.61, 1.07 ± 0.25, and 1.11 ± 0.50 respectively. The mean numbers of flowers per cluster were 1.38 ± 0.89, 2.96 ± 2.30, and 10.57 ± 8.78 (Table 1.2). With respect to the number of flower stems per cluster, ANOVA did not indicate a difference among the three latitudes ($F_{2,733}$=0.5912, P=0.5539, Multiple R-Squared=0.001611), however there was a significant difference in the number of flowers per cluster ($F_{2,654}$=905.9, P<2.2e-16, Multiple R-Squared=0.7348) with increasing flower number at more southern latitudes.

Asexual and Sexual reproduction: Principal Component Analyses (PCA). To examine the shift from investment in sexual to asexual reproduction across latitude, we combined three characteristic indicative of increased investment in asexual or sexual growth and performed a principal component analyses. For sexual investment, a PCA was performed on the traits flower height, flower number, number of flowers per stem and number of flowers per cluster to assess variation in sexual reproductive effort. . For this analysis, the first two principal components explained 98% of the data. For asexual growth, we extracted the first two principal components from an analysis of the traits: number of rosettes in the cluster, cluster diameter and number of rosettes per flowering stem. A PCA collapses the information regarding these three indexes of asexual growth into two components: the first and second principal components which collectively accounted fro 99% of the variation in the data. A plot of the first against the second principal component for both sexual and asexual investment reveals the geographical clustering of individuals by latitude in characteristics relevant to sexual and clonal growth (Figure 1.2).

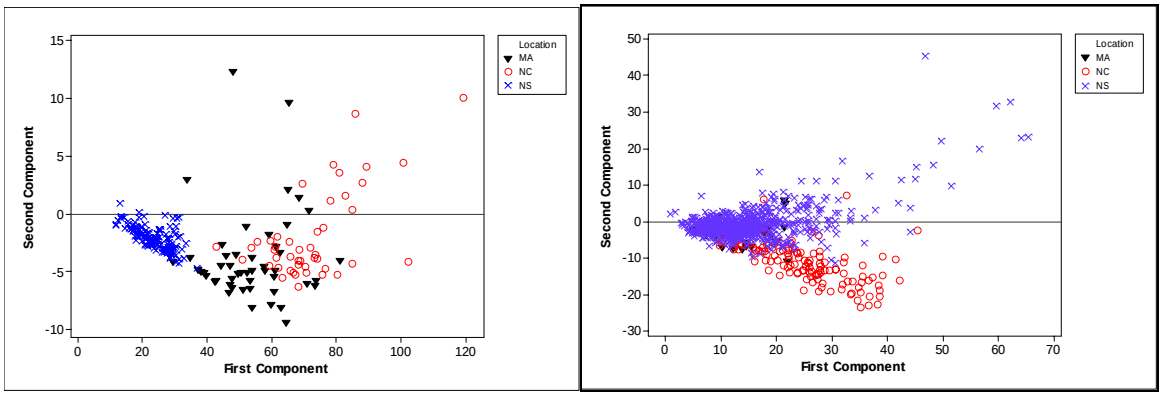

Figure 1.2 Plot of the first against the second principal component extracted from data on sexual (left) and asexual (right) growth.

Difference in morphological, genetics and spatial genetic structure of disturbed versus non-disturbed sites in Nova Scotia.

We identified no differences in morphological characteristics between disturbed and non-disturbed populations in Nova Scotia (Table 1.1). There were significant differences in character traits between lakes and significant lake by disturbance interactions, but no clear trend that morphological characters were influenced by disturbance (Table 1.2, Figure 1.3).

Table 1.1. Summary statistics for morphological characters. P = Pearl Lake undisturbed site, PD = Pearl Lake disturbed site, G = Gillfillan Lake undisturbed site, GD = Gillfillan Lake disturbed site, W = Wilson's Lake undisturbed site, WD = Wilson's Lake disturbed site.

		P	PD	G	GD	W	WD	Pearl	Gill-fillan	Wil-son's	Undisturbed	Disturbed
Flower stem height (cm)	**n**	150	73	95	148	136	81	223	243	217	381	302
	Min	8.2	10.8	15.0	7.6	10.6	14.2	8.2	7.6	10.6	8.2	7.6
	Med.	21.0	20.8	26.5	20.2	24.3	24.4	21.0	22.8	24.4	23.5	21.5
	Mean	20.9	20.8	26.5	20.9	24.6	25.1	20.9	23.1	24.8	23.6	22.0
	Max	30.0	28.4	38.1	33.5	37.0	39.5	30.0	38.1	39.5	38.1	39.5
	SD	3.8	3.8	4.5	4.7	5.1	5.0	3.8	5.3	5.1	5.0	4.9
No. of flowers per stem	**n**	184	73	92	147	136	80	257	239	216	412	300
	Min	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Med.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Mean	1.0	1.0	1.3	1.1	1.1	1.2	1.0	1.2	1.2	1.1	1.1
	Max	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
	SD	0.2	0.2	0.6	0.4	0.4	0.5	0.2	0.5	0.5	0.4	0.4
No. of flowers per cluster	**n**	120	50	61	67	54	21	170	128	75	235	138
	Min	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Med.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Mean	1.1	1.3	1.3	1.3	1.1	1.6	1.2	1.3	1.2	1.2	1.3
	Max	3.0	6.0	4.0	3.0	2.0	3.0	6.0	4.0	3.0	4.0	6.0
	SD	0.4	0.9	0.6	0.5	0.2	0.7	0.6	0.6	0.5	0.5	0.7
No. of flower stems per cluster	**n**	120	50	61	67	54	21	170	128	75	235	138
	Min	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Med.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Mean	1.1	1.3	1.0	1.1	1.0	1.1	1.2	1.1	1.1	1.1	1.2
	Max	3.0	4.0	2.0	3.0	2.0	2.0	4.0	3.0	2.0	3.0	4.0
	SD	0.4	0.8	0.2	0.4	0.1	0.4	0.5	0.3	0.2		
Rosette no. per cluster	**n**	128	50	74	65	70	25	178	139	95	272	140
	Min	1.0	1.0	1.0	1.0	1.0	4.0	1.0	1.0	1.0	1.0	1.0
	Med.	6.0	6.0	5.0	4.0	5.0	10.0	6.0	4.0	6.0	5.0	6.0
	Mean	6.9	8.0	5.0	5.1	6.3	11.5	7.2	5.0	7.6	6.2	7.2
	Max	29.0	31.0	13.0	19.0	29.0	28.0	31.0	19.0	29.0	29.0	31.0
	SD	4.8	6.4	2.7	3.9	4.5	5.8	5.3	3.3	5.4	4.3	5.7
Cluster diameter (cm)	**n**	119	47	68	59	65	25	169	127	90	255	131
	Min	3.6	1.9	3.3	4.4	2.8	8.4	1.0	3.3	2.8	1.0	1.9
	Med.	12.3	10.9	10.0	10.5	11.7	18.6	11.5	10.3	13.2	11.3	11.5
	Mean	13.2	11.2	10.1	10.9	12.4	19.4	12.5	10.5	14.4	12.1	12.7
	Max	30.3	25.8	18.8	32.0	25.0	35.6	30.3	32.0	35.6	30.3	35.6
	SD	5.7	4.8	3.5	4.8	4.6	6.3	5.5	4.1	6.0	5.1	6.0

Table 1.2. **One-way nested ANOVAs to test for the effects of lake, and site within lake on morphological characters.**

Variable	**Source**	**DF**	**SS**	**MS**	***F***	***p***
Flower stem height (cm)	Lake	2	1681.5	840.7	41.3	**< 0.001**
	Lake:site	3	1818.1	606.0	29.8	**< 0.001**
	Residuals	678	13799.8	20.4		
Flower number per stem	Lake	2	1.2	0.6	11.2	< 0.001 *
	Lake:site	3	0.7	0.2	4.0	0.008 *
	Residuals	707	39.0	0.1		
Total flowers per cluster	Lake	2	0.2	0.1	1.0	0.358 *
	Lake:site	3	1.9	0.6	6.5	< 0.001 *
	Residuals	367	35.5	0.1		
Total number of flower stems per cluster	Lake	2	0.6	0.3	3.3	0.037
	Lake:site	3	0.6	0.2	2.3	0.077
	Residuals	418	34.6	0.1		
Total rosettes per cluster	Lake	2	9.5	4.7	9.5	**< 0.001**
	Lake:site	3	11.8	3.9	7.8	**< 0.001**
	Residuals	406	202.9	0.5		
Custer diameter (cm)	Lake	2	4.1	2.0	10.5	< 0.001 *
	Lake:site	3	5.2	1.7	9.0	< 0.001 *
	Residuals	380	73.3	0.2		

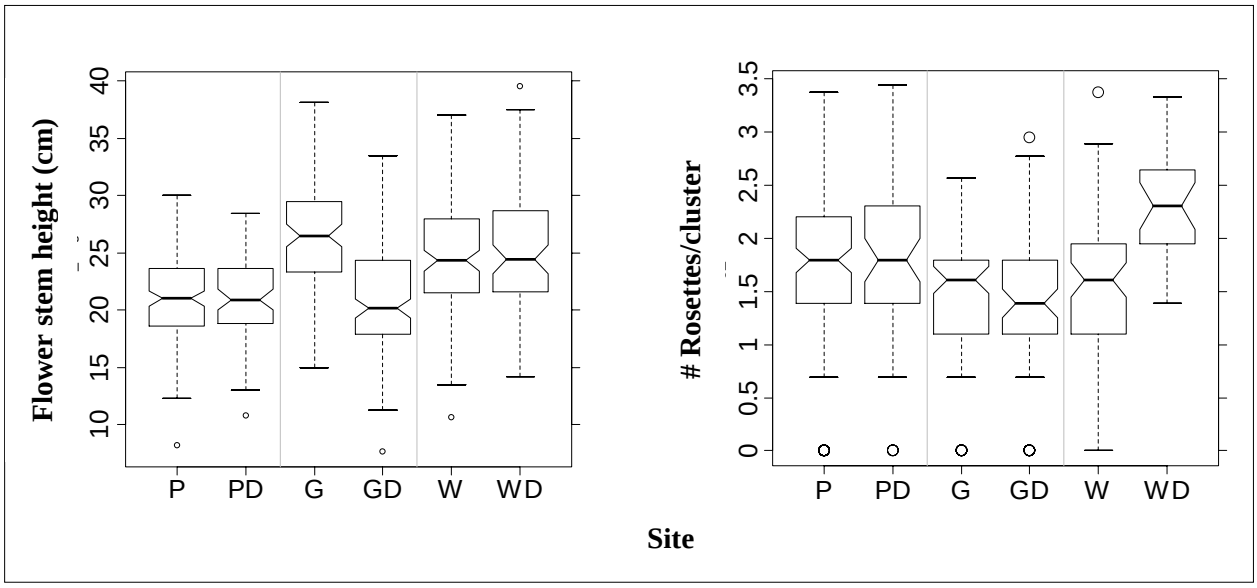

Figure 1. 1. Box and whisker plots of the effect of site on flower stem height (left), and the number of rosettes per cluster (right; data log-transformed). Horizontal lines within the boxes represent median values. Whiskers show the maximum and minimum values except in the case of extreme outliers, which are represented by open circles. Boxes contain the middle 50% of the data (the interquartile range). Boxes for which the notches do not overlap are significantly different at 5% (Crawley, 2005). P = Pearl Lake undisturbed site, PD = Pearl Lake disturbed site, G = Gillfillan Lake undisturbed site, GD = Gillfillan Lake disturbed site, W = Wilson's Lake undisturbed site, WD = Wilson's Lake disturbed site.

Additionally, there were no significant differences in the levels of standing genetic diversity between disturbed and non-disturbed populations of SAKE although there was a trend for that disturbed populations had similar or higher genetic diversity to undisturbed sites (Figure 1.4).

Table 1. 3. Genetic diversity statistics for single populations of parents and seeds from 93 ISSR loci. *na* = total allele number, *ne* = effective allele number, *H* = gene diversity, P = number of polymorphic loci, %P = percentage of polymorphic loci,

		Parents						Seeds					
93 Loci		**P**	**G**	**W**	**PD**	**GD**	**WD**	**P**	**G**	**W**	**PD**	**GD**	**WD**
N		/	30	41	/	35	32	19	15	20	20	20	20
Na	**Mean**	/	1.52	1.48	/	1.56	1.33	1.30	1.20	1.35	1.30	1.22	1.30
	SD	/	0.50	0.50	/	0.50	0.47	0.46	0.41	0.48	0.46	0.41	0.46
Ne	**Mean**	/	1.21	1.15	/	1.21	1.14	1.15	1.11	1.15	1.17	1.10	1.14
	SD	/	0.29	0.26	/	0.31	0.26	0.29	0.26	0.27	0.31	0.24	0.28
H	**Mean**	/	0.14	0.10	/	0.13	0.09	0.09	0.07	0.09	0.10	0.06	0.09
	SD	/	0.17	0.14	/	0.17	0.15	0.16	0.14	0.15	0.17	0.13	0.15
P		/	48	45	/	52	31	28	19	33	28	20	28
% P		/	51.6	48.4	/	55.9	33.3	30.1	20.4	35.5	30.1	21.5	30.1

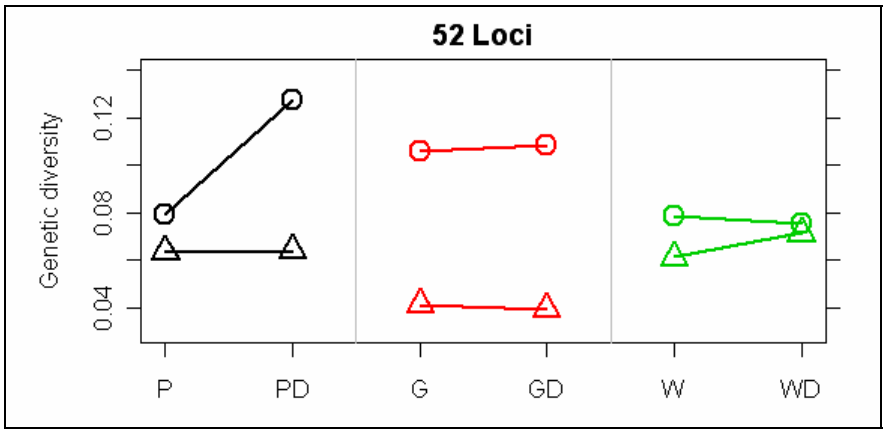

Figure 1. 2. Mean genetic diversities using 52 loci. Open circles represent parent data, while open triangles represent seed data. Sites are indicated along the x-axes, and panels separate lakes. Lines joining circles or triangles indicate changes in genetic diversity between undisturbed and disturbed sites. Panels separate lakes with Pearl Lake on the right, Gillfillan Lake in the middle and Wilson's Lake on the right. P = Pearl Lake undisturbed site, PD = Pearl Lake disturbed site, G = Gillfillan Lake undisturbed site, GD = Gillfillan Lake disturbed site, W = Wilson's Lake undisturbed site, WD = Wilson's Lake disturbed site.

There were, however, differences in the genetic diversity held in each population and, in particular, the population on Pearl was quite distinct from the remaining populations (Figure 1.5) and the partitioning of the genetic variation tended to follow the geographical separation of populations (Fig. 1.5). The observation of marginally more genetic variation in non-disturbed than disturbed populations is evident on the Score plot of the PCA analyses in that the dispersion of points representing individuals from disturbed populations appears greater than that on non-disturbed sites (Fig 1.5).

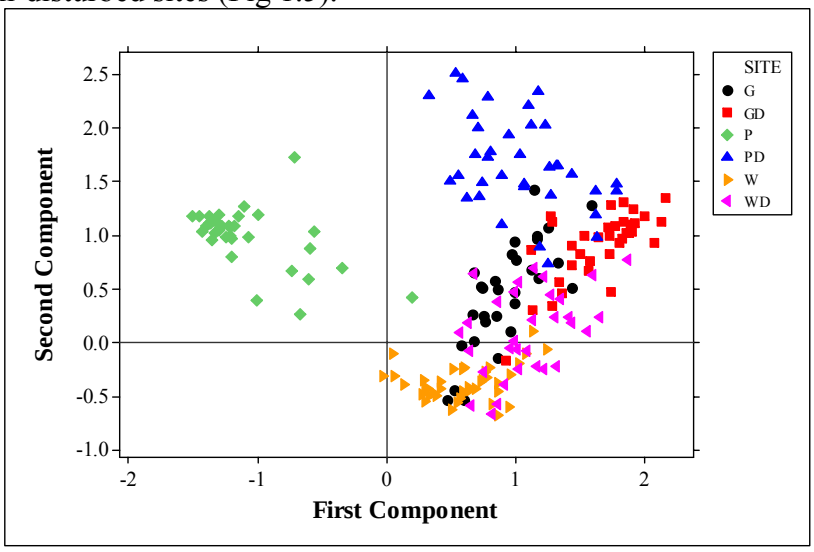

Figure 1.5. Score plot of Parent ISSR data. Individuals were coded for site. P = Pearl Lake undisturbed site, PD = Pearl Lake disturbed site, G = Gillfillan Lake undisturbed site, GD = Gillfillan Lake disturbed site, W = Wilson's Lake undisturbed site, WD = Wilson's Lake disturbed site.

This is further substantiated by the analyses of the decay in genetic relatedness as a function of physical distance across the shoreline analysed showed that there was a significantly negative decline in genetic relatedness with increasing physical separation (Table 1.4). More interestingly, there was a significant difference in the decline in relatedness over distance in disturbed versus non-disturbed sites with the overall relatedness showing a lower level and a lower change in disturbed compared to non-disturbed sites (Fig. 1.6). The genetic relatedness of pairs of individuals decreased from approximately 0.15 in undisturbed and 0.05 in disturbed sites for neighbouring individuals to ~ -0.05 in undisturbed and -0.025 in disturbed sites for individuals ~ 25 m apart (Fig 1.6). Cumulatively, this shows that disturbance is changing the spatial genetic structure of populations either by reducing the amount of asexual reproduction, and thereby decreasing the extent of clonal propogation, and/or favouring the establishment of seeds from unrelated individuals (in the same or different populations). This is consistent with the non-significant trend of greater genetic diversity in disturbed sites and collectively shows that disturbance is affecting the genetic structure of SAKE populations.

Table 1.4. Results of regression analysis to test the effect of the logarithm of distance between pairs of individuals on kinship.

Source	**DF**	**SS**	**MS**	***F***	***p***
Regression	1	0.0739	0.0739	44.13	< **0.001**
Residual Error	58	0.0971	0.0017		
Total	59				
multiple r^2 = 0.432					

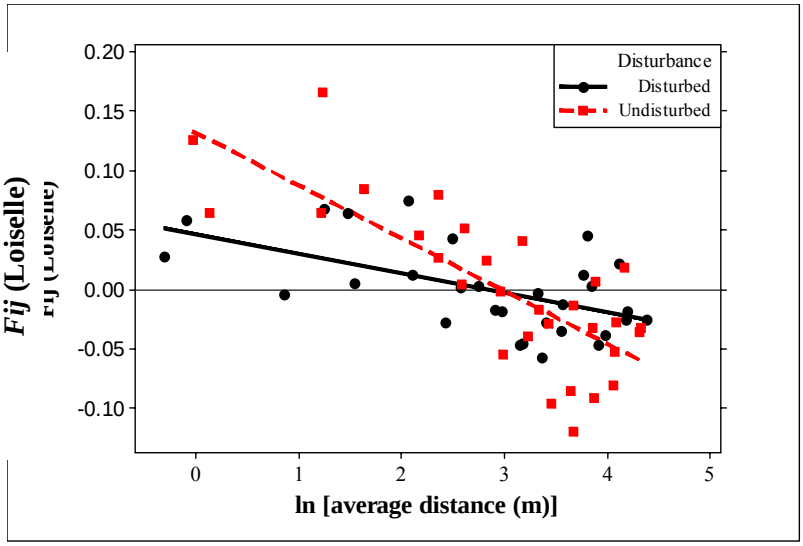

Figure 1.6. Scatter-plot with regression of Loiselle kinship coefficients (*Fij*) against the logarithm of the average distance (m). Points are grouped by disturbance. Solid line is regression fit for disturbed sites, dashed line is regression fit for undisturbed sites.

Summary of goals achieved. In this part of the project, we have shown that there is a change in the relative investment in asexual versus sexual growth across the range of this species. There is strong evidence that investment in sexual reproduction is greater in North Carolina than in Nova Scotia or Massachusetts. In addition, we observed a large number of bumblebees in North Carolina visiting flowers whilst this is a rare occurrence in Nova Scotia

suggesting that there has been a shift in pollinator over the range of the species. This will be discussed more in the final summary. We have also found that while there are no differences in morphology between disturbed and non-disturbed sites in NS, there is evidence that disturbance is disrupting the spatial genetic structure of populations specifically, by favouring the establishment of unrelated individuals (probably via seed) in disturbed sites.

Goals Achieved. The field work, analyses and much of the manuscript writing has been completed for this section. However, we were not able to complete the section on inbreeding depression in SAKE (Objective 3 under reproductive biology of SAKE) because of a failure of the seeds to germinate in the growth chamber.

Section II. Reproductive Biology of *Coreopsis rosea*

a. Determine self-incompatibility SI status by detailed comparison of pollen germination from hand self, crossed and open pollinations. Determine the presence and extent of such processes that promote genetic diversity within populations determine if such systems are breaking down in fragmented and disturbed populations, and thus contributing to loss of genetic variation within these populations.
b. Look for variation in self-compatibility within NS and Massachusetts (MA).
c. Determine if seed production in CORE is limited by a paucity of pollen receipt or mate compatibility by performing hand-pollinations among individuals both within and between populations.

Background

If populations of plants are small and fragmented, then geographically or ecologically isolated populations may diverge from central populations both morphologically and genetically as a result of genetic drift and natural selection (Frankham et al. 2002). Plants, being sessile and dependent on microhabitats for establishment and growth, are particularly prone to developing a patchy population structure. The mating system and mechanisms of pollen and seed dispersal of a plant species will influence how it responds to changes in size and patchiness. When population size is reduced and patchiness increased in populations of plants that have self-incompatibility systems, this can greatly reduce the recruitment of offspring from sexual recruitment because individuals may not encounter compatible mates. This arises because species that are self-incompatible require cross-fertilization from an individual with a different self-incompatibility mating type. If the number of individuals in a population is low, then plants possessing self-incompatibility systems will be less likely to encounter a compatible pollen donor because populations lose diversity at the S-locus. Studies in other plant species in the family Asteraceae, have shown that the presence of self-incompatibility in these species, has limited seed set and contributed to an increased risk for extinction (Les et al. 1991, Reinartz and Les 1994, Gibblin and Havillon 1999, Young et al. 2002). However the deleterious effects of small population size can reach beyond difficulties in encountering compatible mates. Deleterious effects can also arise because plants that have self-incompatibility systems are outbreeders and maintain relatively high levels of genetic diversity but also genetic load. When such populations become small, individuals are forced to inbreed and their progeny are expected to become homozygous for deleterious alleles leading to offspring with poor overall vigour or fitness, a phenomena called inbreeding depression. Biparental inbreeding (inbreeding not caused by self-fertilization) in self-

incompatible species can have dire consequences for both vegetative and reproductive traits in plants (Vogler et al. 1999, Good-Avila et al. 2003) which could further increase the risk of extinction of these species.

To understand the impacts of self-incompatibility, genetic drift (as a function of population size) and inbreeding depression, we undertook a study to examine the a) presence of self-incompatibility b) the effect of population size on pollen load of natural populations and c) the effect of inbreeding and outbreeding depression in the female fertility in Nova Scotian and US populations of CORE.

Methods

Study Organism and Range

Coreopsis rosea Nutt. is the most endangered member of the Atlantic Coastal Plain Flora. Both COSEWIC and the Nova Scotia Endangered Species Act (NS ESA) list CORE as an endangered species. *Coreopsis rosea* has an International Global Rank of G3 meaning that this species is rare and at risk of extinction in its range (Elderkin et al., 2004). Nova Scotia populations of CORE are threatened by habitat fragmentation and destruction as a result of human lakeshore activity. *Coreopsis rosea* populations are often destroyed as a result of cottage construction and development. Shoreline foot traffic and all-terrain vehicle use frequently disturb CORE habitat and hydro-electric damming has resulted in the periodic flooding of CORE populations (Keddy, 1985). C*oreopsis rosea* belongs to the Asteraceae family. It is a perennial herb with 5-8 rosaceous ray flowers and 60-120 yellow disk flowers. It blooms from late July to mid-September. Its fruits are winged achenes. Like other members of the ACPF, CORE inhabits gently sloping, gravelly shorelines of lakes or ponds (Smith, 1976). The range of CORE extends along the eastern coast of the United States from Massachusetts to Maryland. Disjunct populations of CORE are found in South Carolina and southwest Nova Scotia (Cosner and Crawford, 1994). *Coreopsis rosea* has 13 chromosomes and is reported to be self-incompatible (Smith, 1976). Of the 132 species in the genus *Coreopsis*, 19 species are self-incompatible, 1 species is partially self-compatible, and 1 species is self-compatible (Smith, 1976; M. Ferrer and S.V. Good-Avila, in press). The mating systems of the other species in this genus have not been described. *Coreopsis rosea* is also capable of reproducing clonally (Smith, 1976).

Sampling locations (Canada and USA). Nova Scotian populations.

In August of 2005 we identified wild populations of *Coreopsis rosea* in Nova Scotia on 5 lakes: Wilson's, Bennetts, Raynards in the Tusket river watershed and at Salmons and Sloan's on the Annis River watershed (Figure 2.1). On these lakes 9 study sites were identified and field work was conducted on 8 out of the 9 sites, excluding Raynards lake which is a dammed lake. In 2006 we identified populations of CORE on six ponds in Massachusetts in the Cape-Cod-Plymouth area (Figure 2.1b). Some of our experiments on CORE were conducted in the field and others were conducted in the greenhouse at the K.C. Irving Environmental Center on plants grown from seed (NS) or rosettes (US populations). Initially, we intended to do more experimental work in the field, but in both 2005 and 2006 populations of CORE were flooded on most lakes during the flowering period. In 2005, flooding was caused by repair to a dam on the Tusket River watershed. In 2006, flooding was caused by heavy rain. In 2005, the study site on Salmon lake was extirpated by a cottage owner who had recently moved to the area and opened a beach on his waterfront. This

provoked us to find a new study site on Salmon's Lake (which was good since the sites were unknown to DNR) and provided opportunity for education and stewardship.

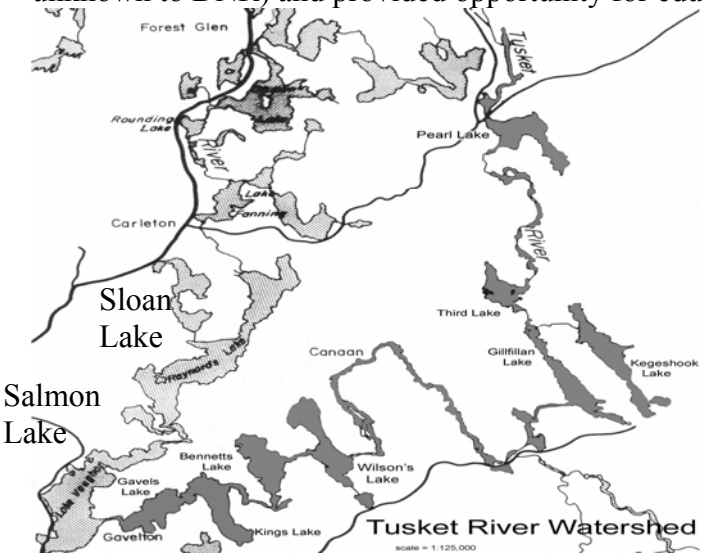

Figure 2.1 a Map of the distribution of studied Nova Scotian populations of CORE.

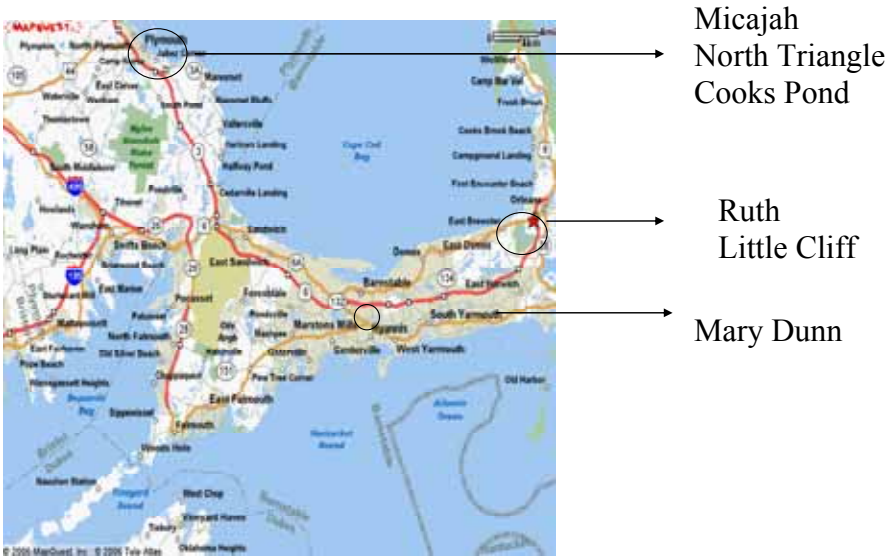

Figure 2.1 b Map of the distribution of populations of CORE sampled from Massachusetts

Breeding system and incompatibility system of Coreopsis rosea

Plants presenting sporophytic self-incompatibility 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.

We evaluated the presence of self-incompatibility in CORE in populations from NS by applying 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 MA, 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 the 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. Pistils were placed 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 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 2.2). 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.

Effect of population size on pollen load of natural populations of *Coreopsis rosea*

The effect of population size on the amount of pollen deposited in natural populations was evaluated by 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, N.S. in August, 2005 and at Sloans lake September, 2006. Eight patches with variable

size 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.

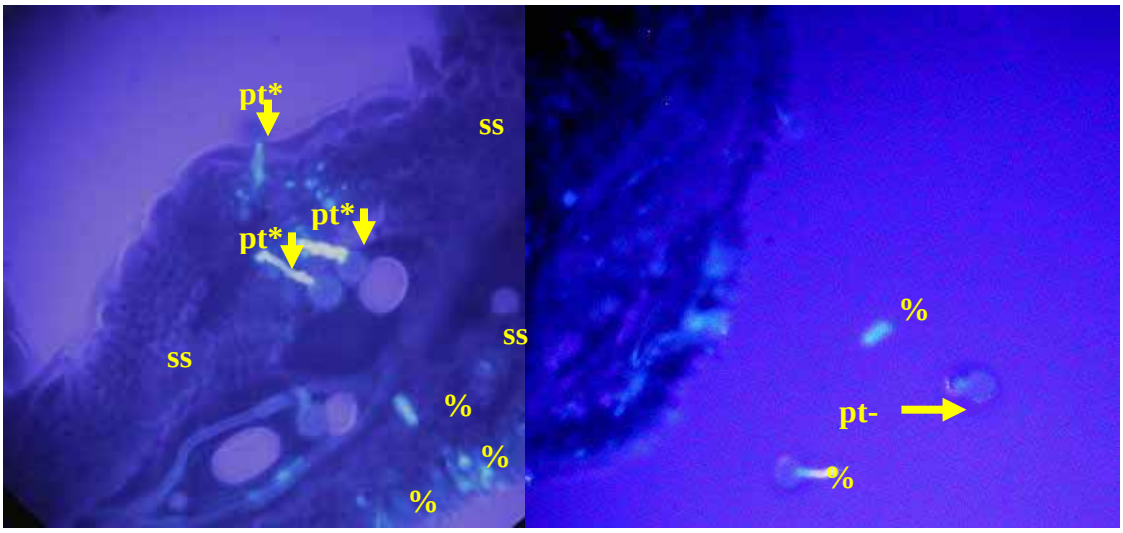

Figure 2.2. Microphotograph of *C. rosea* stigma stained with aniline blue (0.1% KPO_4) and visualized with epi-fluorescence (UV filter). Top. Pollen tubes growing and stopping (indicated with an arrow pt*) before penetrating the stigmatic surface (ss) 9 hr after self pollination. Population Mary Dunn, 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.

Results

The number of flower heads per population at each site in Nova Scotia varied from 5 to 2499 (Table 2.1). Population locations are shown in Figure 2.1a.

Table 2.1. 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)	

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 2.3). The patch with the highest germination and growth was the largest patch consisting of 76 flower heads (Figure 2.3). 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.

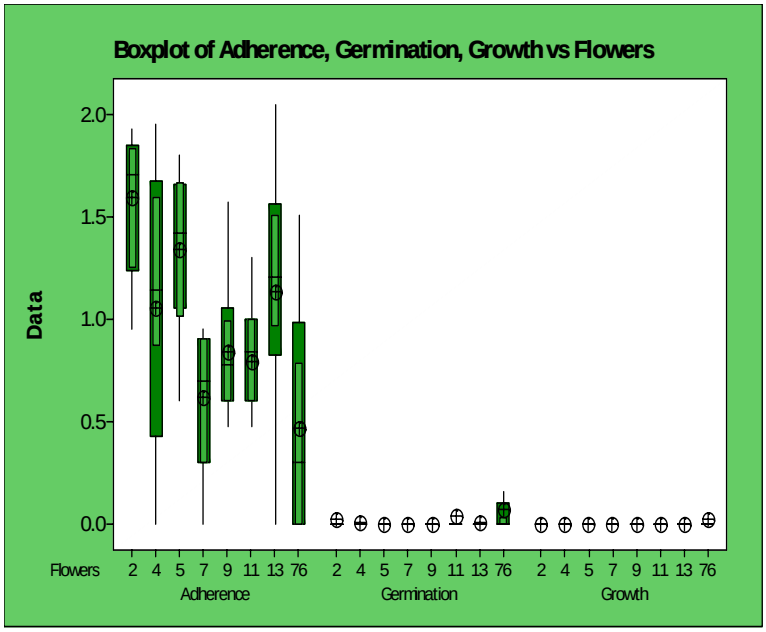

Figure 2.3 Mean number pollen grains that adhered, germinated and grew into styles for individuals from eight different patches of *Coreopsis rosea* growing at Salmons Lake, Canada.

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 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.

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 chapter 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.

Section III: Conservation Genetics of *Sabatia kennedyana* and *Coreopsis rosea*

Summary of Objectives as listed in grant: 1)Conservation genetics in SAKE: Complete analyses of genetic diversity and gene flow among NS and MA populations. Determine genetic variation within and among populations in disturbed and undisturbed habitats to determine if genetic diversity is decreased within fragmented populations and if genetic drift is increased among these same populations. 2) *Conservation Genetics in CORE:* Determine the genetic structure, levels of inbreeding and gene flow among populations of CORE in NS, and MA.

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 historical biogeography and current population genetic structure of two species of Atlantic Coastal Plains Flora, *Sabatia kennedyana* (SAKE) and *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* and *Sabatia kennedyana*

Leaf material was obtained from 4 lakes in Nova Scotia and 3 ponds in Massachusetts for CORE and for 7 lakes in Nova Scotia and 5 ponds in Massachusetts for SAKE (Table 3.1 and Figures 2.1and b). 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 3.1. Coordinates (latitude and longitude) of populations of *Sabatia kennedyana (SAKE)* and *Coreopsis rosea* (CORE) in southwest Nova Scotia (NS) and Massachusetts (MA) (Google Earth, 2006).

Population	Species	Coordinates
Pearl	SAKE	N/A
Third	SAKE	N/A
Kegeshook	SAKE	N/A
Gillfillin	SAKE	N/A
Lac D'ecole	SAKE	N/A
Bennetts, NS	CORE, SAKE	43°55'42.15"N 65°54'10.21"W
Wilsons, NS	CORE, SAKE	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,SAKE	41°40'30.39"N 70°16'44.63"W
Cooks Pond, MA	CORE,SAKE	41°55'20.40"N 70°39'54.75"W
Harlow Pond, MA	CORE,SAKE	41°55'11.94"N 70°40'12.58"W
Denrock	SAKE	N/A
North Triangle	SAKE	N/A
North Carolina	SAKE	N/A

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

The DNA samples of CORE and SAKE 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, while those for SAKE were amplified for two regions, the spacer between trnS and trnG and that flanking rpL (Hamilton, 1999). For the nuclear genetic analyses, the CORE and SAKE DNA 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, more analyses were conducted with SAKE for the chloroplast DNA sequence data. 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. Analyses of the SAKE data set was performed by joining together the two chloroplast DNA sequences to give a total of 1197 base pairs of aligned sequences for a total of 98 individuals from Nova Scotia, Massachusetts and North Carolina. A phylogenetic tree of the relationship among SAKE was

constructed using the Kimura 2- parameter and the minimum evolution optimality criterion as implemented in Mega 3.1. A minimum spanning network was also constructed using the program NETWORK based on the median-joining algorithm for joining sequences.

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 3.2).

Table 3.2. 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

A positive relationship was observed between expected heterozygosity and population size (Figure 3.1). 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 3.2).

The F-statistics show that the overall levels of differentiation of subpopulations in NS is high (S =0.35) (Table 3.3). 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 3.3).

Table 3.3. 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

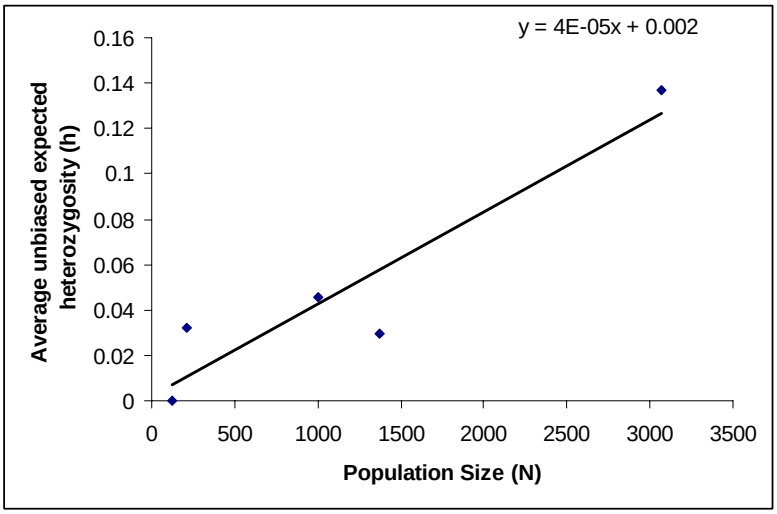

Figure 3.1. Average unbiased expected heterozygosity (*h*) versus population size (*N*) for the *C. rosea* populations in MA and NS populations

The Principal Components Analysis demonstrated that the Mary Dunn Pond individuals had multi-locus fingerprints which were unique from those in Nova Scotia (Figure 3.2), Wilsons Lake formed a loose cluster and Bennets and Wilson's were closely related.

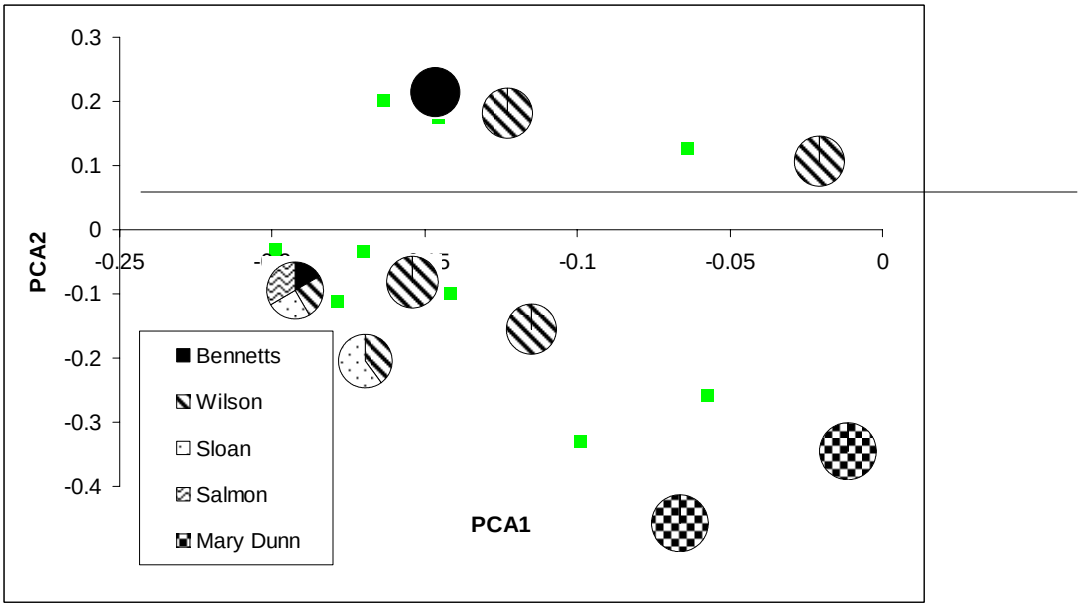

Figure 3.2. Principal Components Analysis performed using ISSR primer 840 data.

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 3.3). 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.

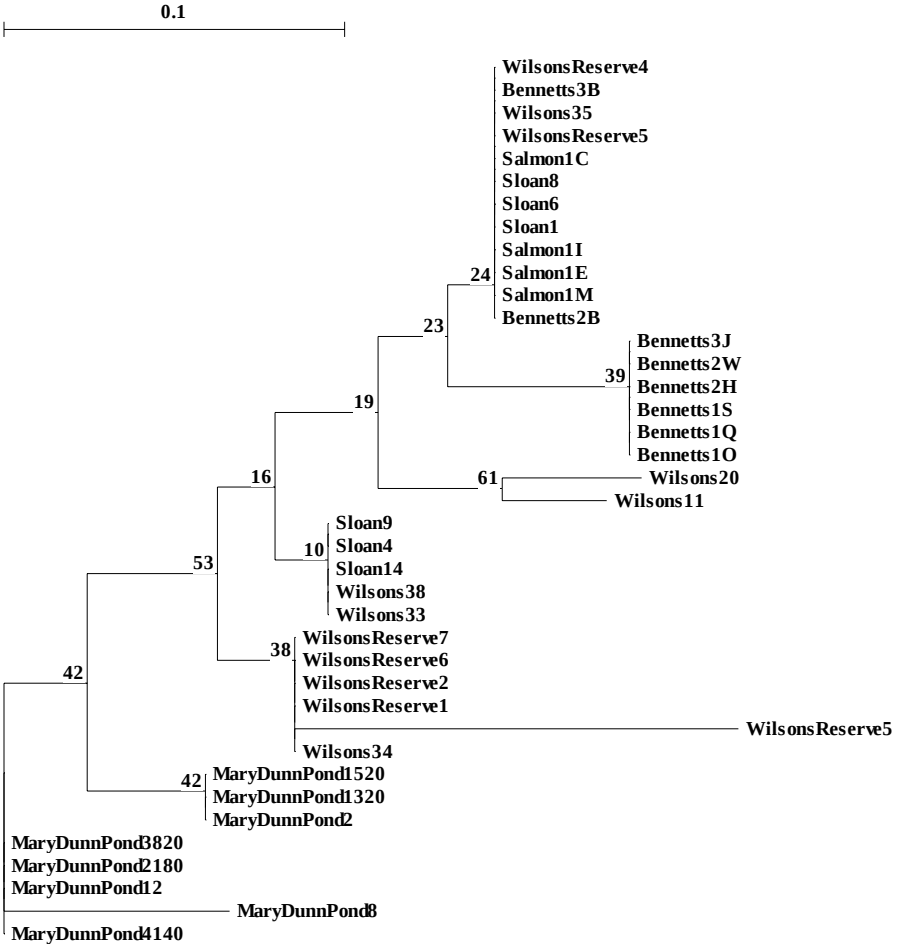

Figure 3.3. Neighbor-joining tree constructed using ISSR primer 840 data for 39 *C. rosea* individuals from Massachusetts and Nova Scotia. Bootstrap values were calculated as percentages of 1000 replications. The tree was rooted using the Mary Dunn Pond sample 4140.

Sabatia Kennedyana

Genetic diversity in populations at both chloroplast and nuclear markers

In total 1197 base pairs of sequence were obtained from 98 individuals of SAKE. Mean nucleotide diversity (theoretical heterozygosity) ranged from less than 0.001 per population to 0.005 per population (Figure 3.4). Genetic diversity was significantly lower in Massachusetts than Nova Scotia for the chloroplast marker (Figure 3.4, middle panel), but

similar for the ISSR marker (Figure 3.4 lower panels). Data are still being collected for two more populations in MA and the populations in NC sampled this year for the ISSR data.

Figure 3.3. 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.

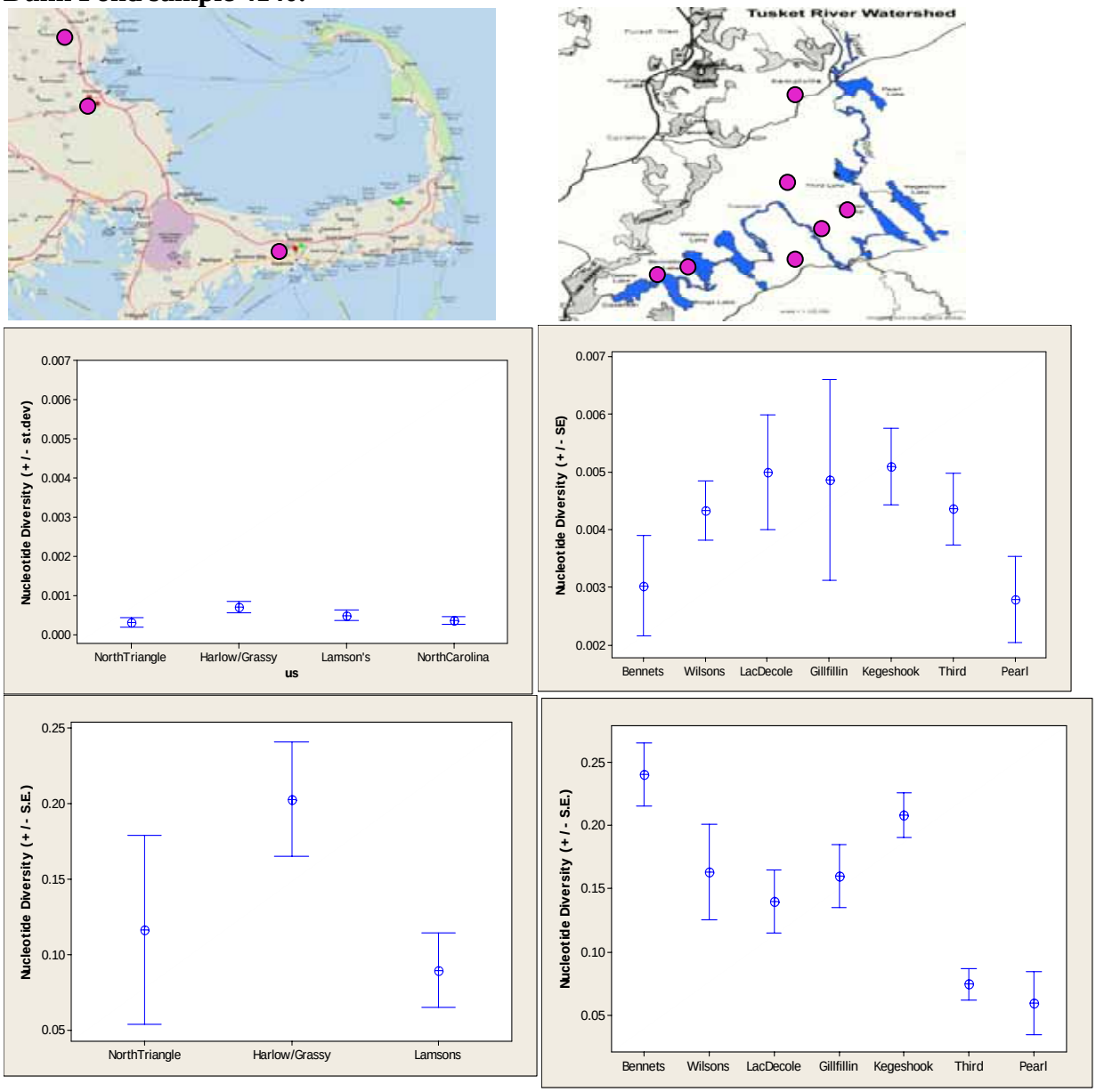

Figure 3.4. Upper panel. Map of the location of populations of SAKE in MA and NS. Middle panel. Nucleotide diversity in corresponding populations for 1197 base pairs of chloroplast DNA sequence data. Lower panel. Nucleotide diversity at ISSR primers for the same populations

Genetic differentiation among populations

The genetic differentiation based on Fst values between populations revealed similar patterns at the chloroplast DNA sequence and the ISSR sequence data although there was

lower overall genetic variation in the chloroplast sequence. A neighbour joining tree based on ISSR data reveals that the US and Nova Scotian populations are differentiated but also that the Nova Scotian populations lowest on the Tusket River water, Wilson's and Bennett's lake populations, are more similar to the US populations than are the populations higher up on the tusket river watershed (Figure 3.5a). A tree based on Fst values derived from the chloroplast DNA sequence data shows a similar pattern but lower overall levels of diversity between US populations including the population in North Carolina (Figure 3.5b). Interestingly, both nuclear and chloroplast markers suggest that populations higher up on the tusket river watershed (coloured as red circles) are more similar to each other than to ones lower down on the tusket watershed (yellow triangles) which are more closely related to Massachusetts populations (green and/or blue squares) (Figures 3.5a versus b).

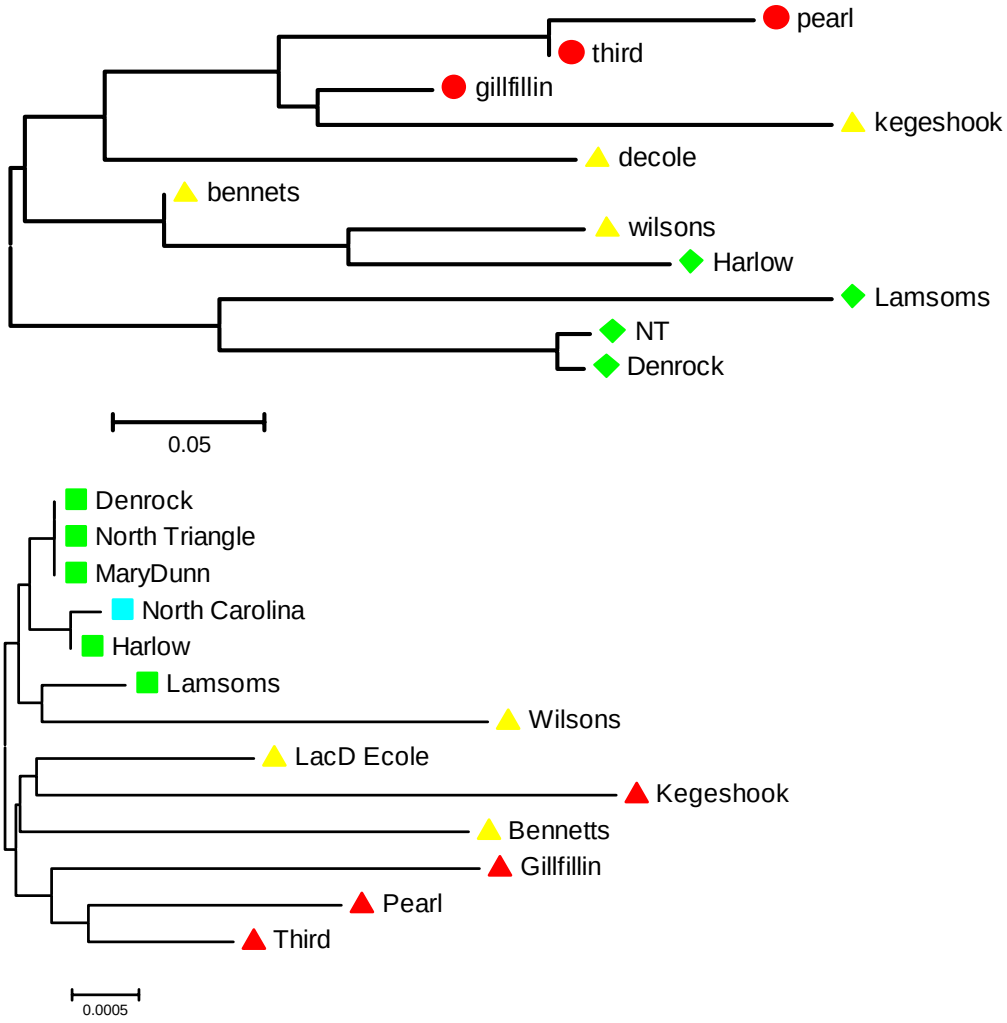

Figure 3.5 Neighbour joining tree based on Fst distances based on a) ISSR markers (above) or b) chloroplast sequence data (below)

Relationship between geographic and genetic distance

A mantel test of the relationship between geographic and genetic correlation shows that there is positive relationship between the two distances for the chloroplast marker but not

based on the nuclear marker (Figure 3.6). Given that connectivity is maintained at chloroplast markers via seed flow and at nuclear markers by both seed and pollen flow, this suggests that seed flow occurs down the watershed in a manner consistent with isolation by distance but that pollen flow mixes up genetic relatedness, probably because pollinator can fly "against" the flow of the water while seeds are dispersed through it.

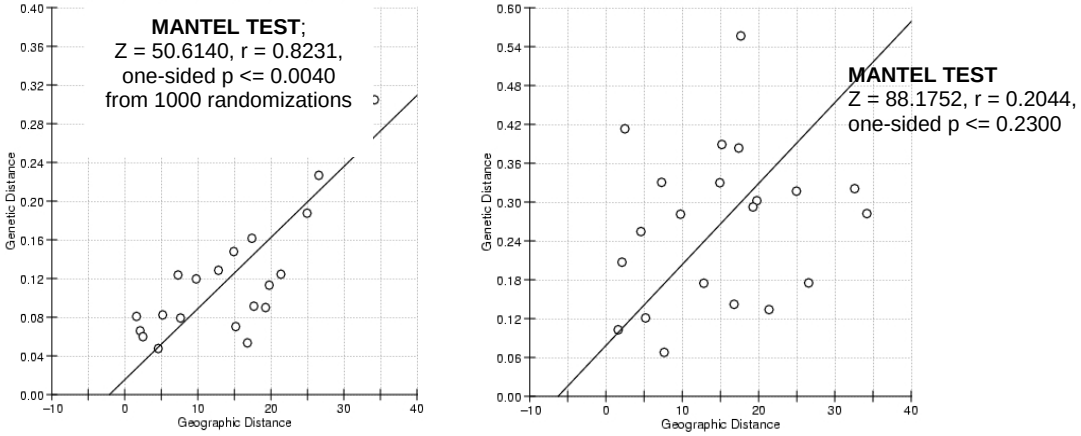

Figure 3.6 Mantel test for relationship of geographic to genetic distances for 7 populations of *S. kennedyana* in N.S. Test shows a positive relationship between geographic and genetic distance based on the chloroplast marker (left side) but not based on the nuclear ISSR markers (right side).

<u>Phylogenetic trees and networks of *S. kennedyana* cpDNA sequence data.</u>

The chloroplast DNA sequence data shows low, but informative levels of DNA polymorphism. The relationship among existing haplotypes is nicely observed on a network tree in which ancestral haplotypes map to the center of the network and derived groups extend from that. On the tree, the length of the branches is proportional to the number of mutations occurring on them (Figure 3.7) The relationship of the haplotypes in the network reveals that haplotypes from the lower tusket, Massachusetts and North Carolina occur in the most central and ancestral positions. However, the haplotypes in this central position are not highly variable. This strongly suggests that all modern populations are derived from one ancestral population, i.e. that there was only one refugium for all modern populations of SAKE. It also shows that Nova Scotian populations harbour these ancestral haplotypes suggesting that the refugium was probably not in North Carolina. In addition, there are two main divergent clades: one corresponds primarily to haplotypes from the upper tusket river water in NS and the other to individuals from either the populations in MA or NC. This suggests that these populations have been isolated from other populations.

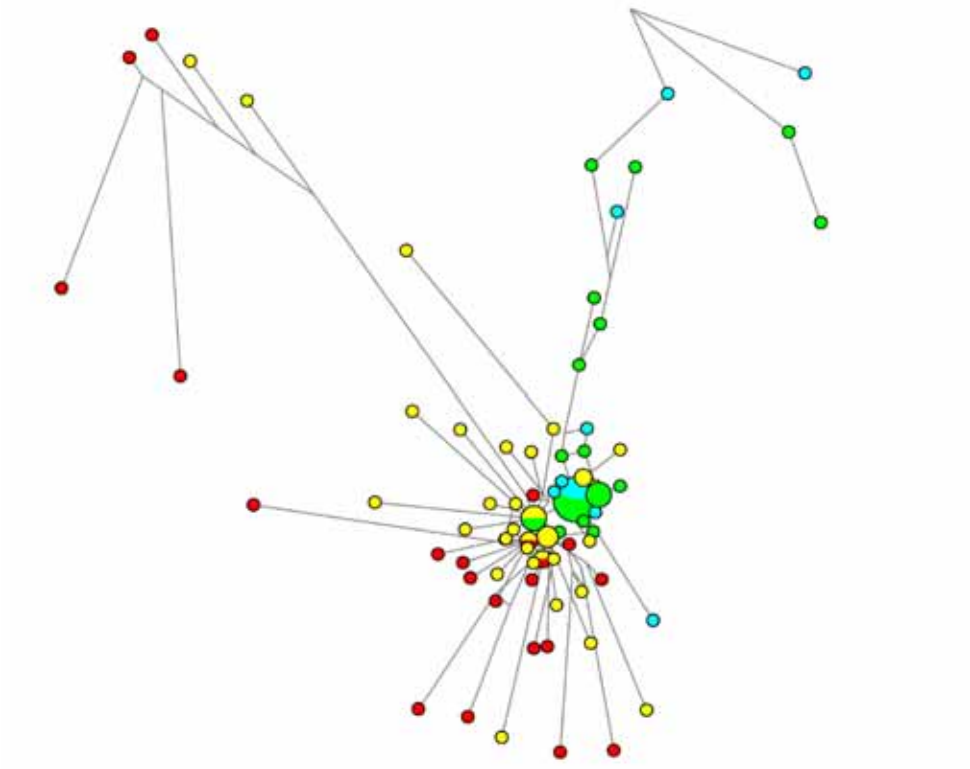

Figure 3.7 Minimum spanning network of haplotypes in *S. kennedyana*. Red refers to haplotypes derived from the upper tusket, yellow – the lower tusket, NS, light blue-North Carolina, green – Massachusetts.

The relationship among the SAKE DNA sequences can also be depicted as a traditional phylogenetic tree (not shown) this reveals a similar trend: one clade consists of primarily upper tusket haplotypes, one of primarily US haplotypes and the third of haplotypes from all three regions. i

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*, this report). 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.

Sabatia kennedyana

The results of genetic data analyses are significantly different for SAKE than for CORE. Firstly, there are higher, overall, levels of genetic diversity in SAKE. SAKE is a self-compatible species that seeds 400-600 seeds per fruit after open-pollination in NS and MA (Trant, 2005, Sutton, unpublished). The plant also grows clonally and there is an increase in asexual, clonal, reproduction in northern populations (section I). Despite this, there is equal or greater variation in the chloroplast DNA sequence data in NS than in MA or NS and similar amounts of variation at the nuclear, and faster evolving, ISSR markers. Collectively our data suggest that there was a single refugium for SAKE during the last glaciation and perhaps more surprisingly that this refugium may have been in the NS – Cape Cod region. Shaw et al. (2002) have recently shown that around 11,000 years ago, large areas of the continental shelf were exposed off of Cape Cod and Nova Scotia. Although there could not have been a continous stretch of land in this region, it is possible that the coastal species were located on islands on the shelf and that their seeds were dispersed via water or birds along the coast. When water levels were at their lowest level, the distance separating the Cape Cod from the Nova Scotian continental shelf would not have been great. Our data analyses in SAKE support the hypothesis that a refugia existed in this area.

The comparison of the genetic structure of populations of SAKE in NS using maternally (chloroplast) versus maternally and paternally (ISSR) inherited markers can reveal different histories of the connectivity of populations via seeds (or entire plants) and pollen flow in natural populations. The mantel test comparing isolation by distance for the maternal versus paternally inherited markers, showed that the maternal marker exhibits significant isolation by distance but the nuclear markers do not. This strongly suggests that connectivity via seeds is maintained by gene flow down the tusket river watershed, but that pollen flow "mixes" up the genetic relatedness of populations within lakes. Although populations are not isolated by distance per se based on the nuclear markers, both the nuclear and chloroplast markers show that genetic variation in SAKE falls into two distinct regions in the tusket river watershed in NS: the populations at the head of the tusket river, Pearl, Third, Gillfillin and Kegeshook, share more closely related genetic diversity than they do to the populations at the base of the tusket river watershed, Wilson's and Bennetts. Interestingly Lac d'ecole, which is quite isolated and lies in the middle of the upper and lower tusket river lakes has high genetic diversity and shares haplotypes from both lower and upper tusket river lakes. Our data also strongly indicate that the populations in the lower tusket river watershed,

Wilson's and Bennetts, share a closer genetic relatedness to MA populations of SAKE than do the populations in the upper tusket. The fact that populations separated by almost 600km (NS to MA) could be as similar to each other as populations (upper to lower tusket) 15 kilometres away shows the power of molecular markers to reveal relationships of historical biogeography.

Overall Summary:-

As mentioned, the genetic and breeding system data are roughly in agreement for CORE. Both the genetic and breeding data suggest that NS populations of CORE may be relying heavily on asexual reproduction and are suffering from the effects of small population size, in particular random genetic drift. Because the species is self-incompatible and requires cross-pollination to set seed, it appears to be even more difficult for the species to reproduce via sexual reproduction. Efforts to collect wild produced seed in CORE by our lab and by other students at Acadia University has shown that populations of CORE at the Wilson's lake reserve and Raynards Lake set some seed, but that mean seed set is very limited in all other populations.

However, the situation in SAKE appears quite different. Our genetic analyses suggest that there are reasonable levels of genetic variation in NS populations of SAKE and, indeed, that Nova Scotian populations harbour an important fraction of the historical diversity of alleles in this species. Although there is significant genetic differentiation among populations of SAKE within NS, the ancestral closeness of NS and MA populations is clear at the molecular level. However, our analysis of the morphological variation in the species across its range tells a different story. Our morphological data show that the species invests more in sexual reproduction at the southern portion of its range in North Carolina and that Canadian populations of SAKE produce extensively asexually and produce only one to two flowers per rosette cluster. There is some evidence that the species suffers from some levels of inbreeding depression (Trant, 2005, Hill, 2006), but both small and large populations and disturbed and non-disturbed populations show similar fruit and seed set (J. Sutton, unpublished data). In addition, Trant et al (submitted) have shown that NS populations are predominantly pollinated by syrphid flies, while we have observed extensive activity of bumblebees in NC populations of the species (J. Sutton, personal observation) and some indication that there southern populations may produce nectar but northern populations do not (J. Sutton, personal observation). Consequently, while the molecular data suggest a close historical relationship of populations of SAKE across their range, the morphological and breeding system data suggest that there are divergent selection pressures operating in the species from north to south.

Section IV: OUTREACH AND EDUCATION

Summary of objectives: - 4 a) To report findings to landowners and to local conservation groups such as TREPA, and ACPF recovery team and to present the findings at a national or International conference. 4b) To present findings in published manuscripts or theses.

Summary of activities: Similar to ecological processes, conservation initiatives and dissemination of acquired knowledge can be addressed at multiple scales. Our initiative focusing 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, 4 additional landowners in 2005, and an additional 4 landowners in 2006. 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), and have recently agreed to write a general article for their newsletter which we will also submit to the World Wildlife Fund 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 2006 Drs. Miriam Ferrer and Sara Good-Avila traveled to Massachusetts to continue our field work on SAKE and to initiate some field work in CORE. For this trip, we met with Paul Somers and the Massachusetts Natural Heritage Museum and we presented to him the results of our research. This led to further support and contacts in MA including the collaboration with a recently graduated PhD. student, Lelia Orrel, who is doing work on SAKE. In the summer of 2006, Ms. Jolene Sutton performed field work in NC on SAKE and developed further contacts with the members of the department of Fish and Game in NC.

In addition to these outreach initiatives with landowners, government officials and activists in the field, we continue to disseminate our work to the academic community. Our manuscript concerning the effect of fragmentation on pollinator visitation rates in SAKE in NS is currently under review in Plant Ecology, and as is evident from this report, we have completed much of our analyses of the conservation genetic structure of SAKE and CORE. We aim to complete the study of the biogeography and genetic structure of SAKE by the spring of 2007 and submit this as a publication to Conservation Biology or Conservation Genetics. Following this, we envision three more manuscripts: one on the reproductive biology and genetics of CORE, one on the variation in morphology and genetics across the range for SAKE, and one concerning the effect of fragmentation on pollen flow also in SAKE. Because we have several manuscripts underway, we are not applying for more funds for the coastal plains project this year as we would like to concentrate on manuscript submission.

Dr. Sara Good-Avila has presented the results of this research in three locations to date: at Acadia University (February, 2006), St. Francis Xavier (October, 2006) and the National University of Mexico (December 2006). 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.

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1986 Panda symbol WWF-World Wide Fund for Nature (also known as World Wildlife Fund)

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