

Conservation genetics and conservation ecology of Atlantic Coastal Plain Flora at risk in southwest Nova Scotia

Final Report For Nova Scotia Habitat Conservation Fund project (2004 granting year)

submitted by Andrew Trant and Dr. Sara Good-Avila, September 2005.

Executive summary

Our research initiative exploring the pollination ecology and seed bank dynamics of high priority Atlantic Coastal Plain Flora was incredibly successful. Both the field and laboratory components of our study were met with great success and in many instances, we exceeded our projected goals. Field data collection on the pollination ecology and inbreeding depression of *Sabatia kennedyana* was completed in the summer 2004. A total of 156 hours of visitation were collected at nine populations and inbreeding depression was examined on at least 20 plants on 9 populations on 3 lakes (total of individual 360 plants). Furthermore, data were collected on floral phenology, pollen removal, and pollen deposition. Preliminary data suggest that pollinator visitation rates are lower on sites adjacent to cottage develop. Our study on the effects of cottage development on the dynamics of the seed bank in the Tusket River watershed is well in progress. We have identified plants in nearly 1000 soil cores collected from the experiment and are currently analysing variation in the abundance or diversity of species between sites. In addition to these projects which were funded through the Nova Scotia Habitat Conservation Fund, we also worked on the conservation genetics of *S. kennedyana* using funds from other agencies (the World Wildlife Fund) and new funding from the N.S. Habitat Conservation Fund (2005 funding year). Preliminary results from these analyses show that the populations of *S.kennedyana* in Nova Scotia show significant genetic structure and that, in general, populations that are further apart on the Tusket River watershed are more distantly related. This past year has also provided us amazing opportunities to work directly with landowners throughout the watershed. Forming and fostering these relationships is paramount for successful conservation. Through rigorous field and lab efforts, many new questions have been raised that are critical to the recovery of this species. **For example, we have found evidence that cottage development significantly decreases pollinator visitation rates. This means that shoreline cottage development is significantly reducing the quality of the habitat for this species because it disrupts the ecological processes required for sexual reproduction.**

1. POLLINATION ECOLOGY

Summary of objectives: - to determine visitation rates and movement of pollinators within populations of *Sabatia kennedyana*, SAKE, in small, large and disturbed populations and to perform controlled pollination treatments in different populations to examine inbreeding depression and reproductive fitness in populations that differ in size or level of disturbance.

Summary of activities: Prior to flowering, we surveyed 6 lakes throughout the Tusket River watershed to select suitable and comparable study sites. Sites with populations of SAKE at 3 levels of classification (large, small, and close to shoreline disturbance) were recorded with GPS and revisited multiple times to monitor development of flowering plants. A total of 9 sites on three lakes were selected and approximately 20 plants were set up for monitoring throughout the flowering season. At each site, data were collected on the number of flowering SAKE, shoreline habitat, surrounding vegetation and proximity to shoreline disturbance. These populations were monitored at least every three days through the entire summer. To monitor pollinator visitation rates, 1x1m plots were established to monitor pollinator activity. For each pollinator visitation session, we recorded wind speed (maximum and average), relative humidity, temperature and cloud cover. We collected a total of 156 hours of pollinator observations which exceeded our initial target of 100 hours. To examine efficiency of pollinator services, we collected 110 stigmas (following anthesis) to count the number of pollen grains deposited by pollinators. We also collected approximately 250 sets of anthers (at all stages of anthesis) to examine pollen removal by pollinators. On the 20 marked plants, a thoughtfully designed pollination experiment was

conducted to examine the amount of inbreeding depression at each site. Furthermore, these marked plants were used to monitor floral phenology throughout the watershed. A total of 330 flowers were followed throughout the season and fruits collected when possible (some were lost due to herbivory and storm events). The collected fruits will be sown to examine inbreeding depression, reproductive fitness, and the role of pollinators in response to varying population size, position in the watershed and proximity to shoreline development.

Currently: We have finished analysing the pollinator visitation data and have found that there are significant differences between visitation rates between sites (large, small, and disturbed). **In particular disturbed sites showed significantly lower pollinator visitation rates than either large or small populations indicating a negative impact of cottage development on plant-pollinator interactions.** In Figure 1 we present a boxplot of the difference in visitation rates between plants in disturbed (by a cottage) or non disturbed (combined small and large populations) sites. The visitation rate was measured in both high and low plot densities of plants.

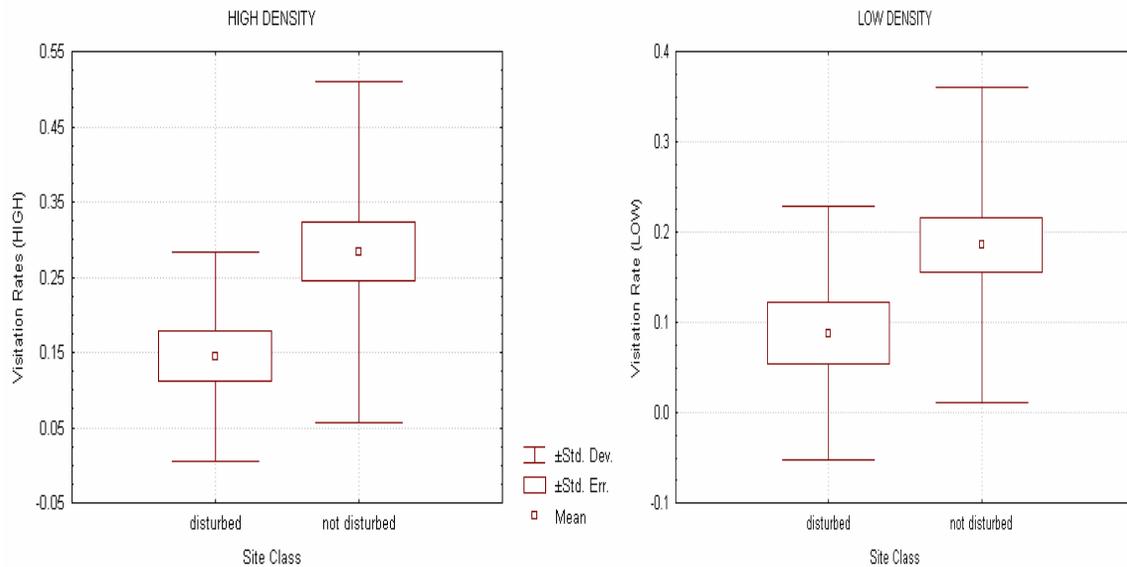


Figure 1. The number of pollinators visiting per observation period in disturbed and non-disturbed habitats and in high (left side) or low (right side) density patches.

In addition, we found that there were no significant differences between populations in the length of time that pollinators visited flowers, **although pollinators in disturbed sites showed consistently shorter handling times than either of the other two site classifications.** This finding is consistent with our finding, presented above, that pollinators visited disturbed sites significantly less than non disturbed sites. Boxplots describing the decrease in handling time of pollinators in disturbed versus non disturbed sites at both low and high density of subplots is shown in Figures 2.

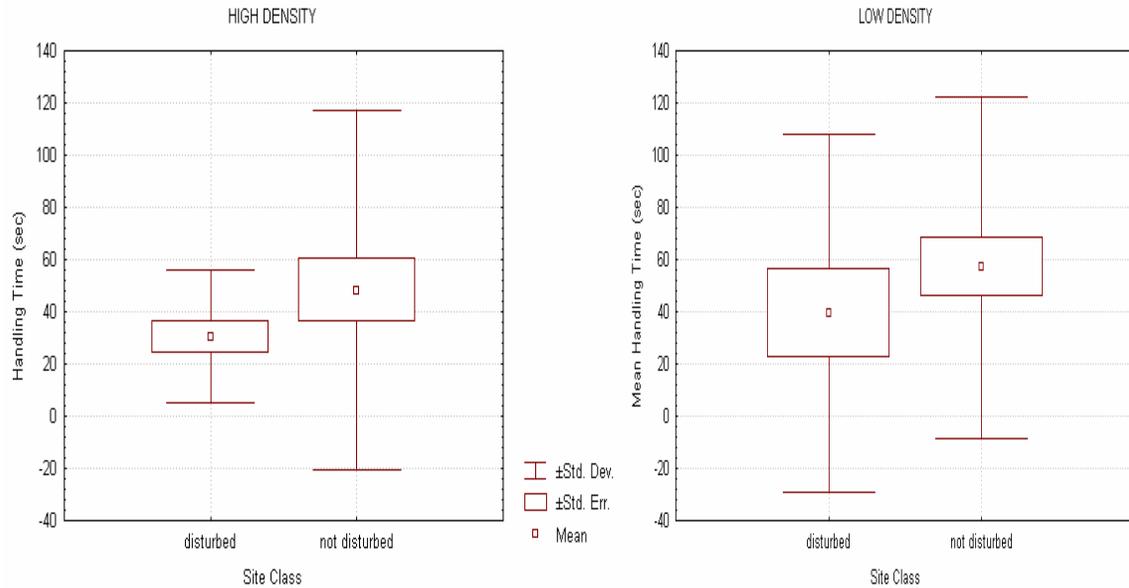


Figure 2. The number of seconds (handling time) that pollinators foraged on flowers of *Sabatia kennedyana* in disturbed and non-disturbed habitats. On the left, the handling time in patches of high density (> 5 flowers patch) and on the right, the handling time in low density patches.

The number and relative percentage of pollinators falling into six pollinator categories is given in Table 1. This reveals that the primary pollinators of SAKE are syrphids. The diversity of pollinators did not differ by habitat, although, surprisingly, Wilson's lake exhibited lower pollinator diversity than either Pearl or Kegeshook lakes. Variation in abundance or diversity of pollinators between habitats and lakes will be studied in future projects.

Pollinators	Number	Percent
Syrphids	1081	66.4
Small syrphid	339	20.8
Halictids (sweat bees)	78	4.8
Large syrphids	46	2.8
Bumblebees (<i>Bombus sp.</i>)	7	0.4
Others	77	4.8

In addition, we found that pollinators were more attracted to flowers in male than female phase probably because the species does not appear to offer nectar.

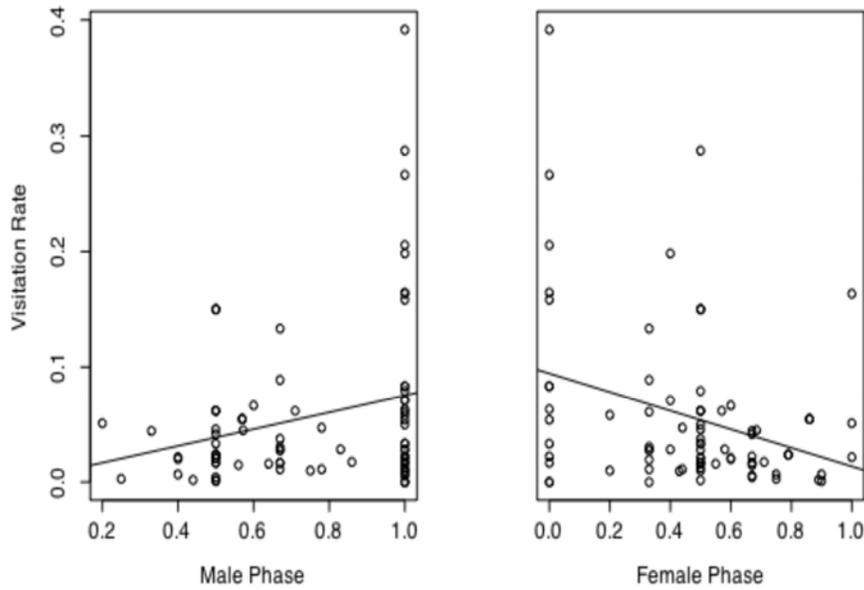


Figure 3. The pollinator visitation rate as a function of the proportion of individuals in a patch in male or female phase.

There was also a slight decrease in pollinator abundance towards the end of the flowering season (Figure 4) and we found that the visitation rate was non-significantly but negatively correlated with the number of other ACPF species flowering at the time (Figure 5).

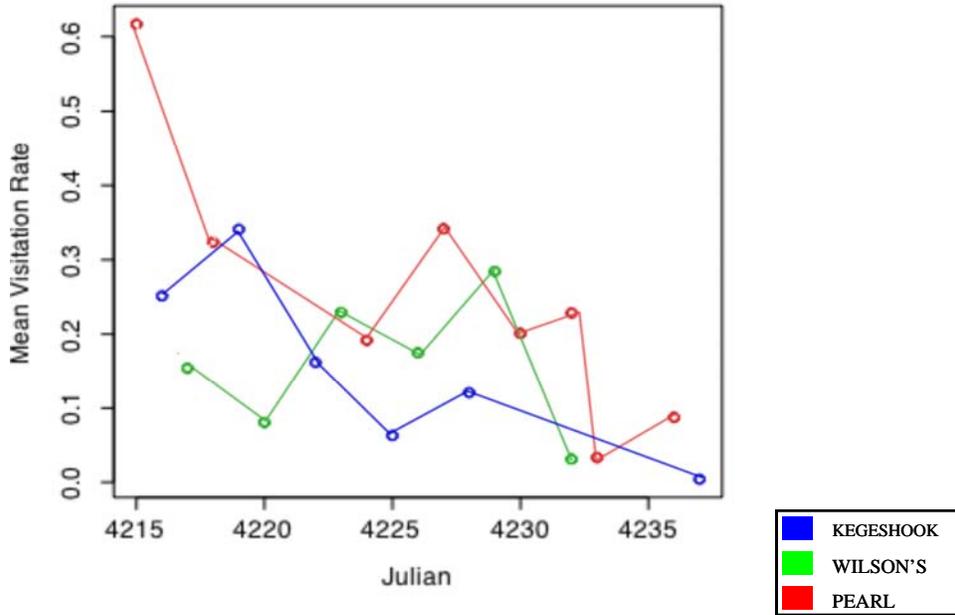


Figure 4. The mean visitation rate of pollinators to populations of *Sabatia kennedyana* on Kegeshook, Wilson's and Pearl lakes over the course of the flowering season in Julian days.

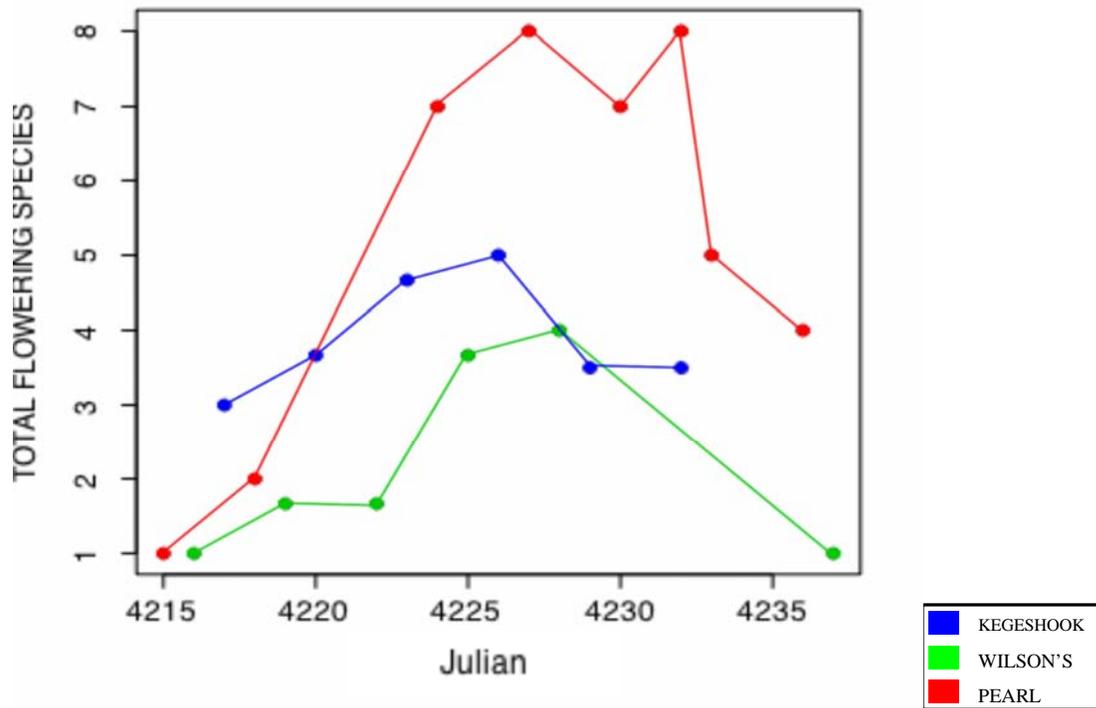


Figure 5. The number of flowering Atlantic Coastal Plains Flora (ACPF) in bloom on Kegeshook, Wilson's and Pearl Lakes during the flowering season of *Sabatia kennedyana* measured in Julian days.

We are currently in the process of growing the seedlings of *Sabatia kennedyana* in the greenhouse that were produced from hand self- and cross-pollinations in the field to examine the interactive effects of population size, level of disturbance and lake on inbreeding depression. This will be completed in about six months.

Goals achieved: All set targets for the pollination ecology portion of the study for SAKE were successfully completed and we have finished all of the analyses for this project except for the analysis of inbreeding depression. The results from the inbreeding depression study will be included in a future manuscript and the completed work on the pollination ecology is currently in preparation for submission to a conservation journal.

2. SEED BANK AND ESTABLISHMENT

Summary of objectives: - to determine the patterns of seed movement and the extent of the seed bank through the Tusket River Watershed and begin investigation into establishment and germination of seeds.

Summary of activities: This portion of the study was conducted on 6 of the 9 sites that were established for the pollination ecology and inbreeding depression study. On each of the 3 lakes, the disturbed sites and the more comparable of the other two sites (either the large or small) were selected. At each site, soil cores (~100cm²) were taken at 5m transects perpendicular to the shoreline for a total of 90m. At each transect, cores were taken starting at the high water line (shrub line) and then every meter towards the water until no terrestrial/semi-aquatic vegetation

was present. In the field, each core was divided an upper and lower strata and were germinated separately to look at the density of seeds in the soil profile (under the assumption that older seeds would be found deeper in the soil). Following collection, the 950 samples were returned to Acadia University where they were dried and sifted to remove all rhizomes that could be confused with germinating seeds (especially relevant for monocots). Soil cores were spread on a 1:1 mixture of sand and potting soil in individual 3"x4" containers and placed in the greenhouse for germination. Cores were watered every third day and monitored weekly for the number of germinating monocots and dicots. Every 3 weeks, the placement of the trays in the greenhouse is randomized to account for the effect of location within the greenhouse. The germinating plants are being grown until identification is possible.

Currently: The soil cores were potted in September 2004 and were maintained in the greenhouse at the KC Irving Environmental Centre until May of 2005. The identification of the plants growing in each core has been completed. The list of species identified in the cores is given below:

Seed Bank Species List

Agrostis perennans L.
Aster tradescanti L.
Betula papyrifera Marshall
Cerastium sp.
Coreopsis rosea Nutt.
Epilobium sp.
Eriocaulon aquaticum (Hill) Druce
Euthamia galetorum Greene
Gnaphalium uliginosum L.
Gratiola aurea Pursh
Hypericum canadense L.
Hypericum perforatum L.
Juncus canadensis J. Gay
Juncus pelocarpus E. Meyer
Juncus tenuis Willd.
Lobelia dortmanna L.
Ranunculus acris L.
Ranunculus repens L.
Rumex acetosella L.
Spergula arvensis L.
Spergula rubra (L.) J. & C. Presl
Spirea sp.
Viola lanceolata L.

Analyses of species number and diversity at sites with different levels of disturbance and at different distances from the focal site are underway. Unfortunately, no *Sabatia kennedyana* were observed in the cores although we did observe another focal species in our laboratory, *Coreopsis rosea*, which is the most endangered member of the ACPF in the Tusket River Watershed.

Goals achieved: The seed bank portion of the study has been a great success! We collected more cores than originally anticipated and preliminary greenhouse results suggest high levels of germination. We are currently conducting statistical analyses to test whether 1) disturbed versus non disturbed sites have a greater abundance or diversity of seed germination 2) there is variation in the diversity or abundance of seeds at different distances out from the focal population (along the transect of the lakeshore relative to the main population where SAKE was

observed and 3) there are differences in the abundance or diversity of seeds on different lakes. In addition, seeds from SAKE and CORE were collected and have passed through stratification by periodic refrigeration. We have established them in growth chambers and are having success germinating both species. We plan to develop greenhouse stocks from all known populations of CORE so that we may continue our studies of the conservation genetics and breeding system of this endangered species in the greenhouse.

3. EDUCATION

Summary of objectives: - to present findings of research 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 in 2004 (as each of our disturbed sites was situated next to a cottage). 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. Recently members of TREPA have provided us with a detailed history of the Tusket River Watershed: we are reviewing this and preparing a document that will include a historical overview of the watershed and integrate our own findings regarding the pollination ecology, seed bank, germination, and impact of cottage development on the SAKE, or the Plymouth Gentian. We have also been attending meeting with 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. Andrew Trant, the Msc. Candidate working on this project, presented results of his work in the Fall of 2004 in Maine, USA at a regional graduate student conference and then in Brasil in July of 2005 at the annual meeting of the Society for Conservation Biology (a well-attended international forum with approximately 2000 participants this year). One-page printouts of the poster's that he presented at these meetings are attached. More recently, Dr. Good-Avila presented a summary of our work on *Sabatia kennedyana* at the first annual meeting of the Atlantic Center for Global Climate Change and Environmental Research (ACGCER). A hand-out of the powerpoint slides presented at this conference is also included. Disseminating acquired knowledge at all of these scales is essential for maximizing the listening audience.

Currently: Communication with TREPA and the ACPF recovery team continues to be opportunistic and continuous. Our results have been presented at one international conference and two regional conferences, but more talks are anticipated included at the N.S. Museum and at regional Universities. In addition, we are preparing a more general article concerning the history of the area and the impact of our findings on future developments to be delivered to TREPA, the NS Habitat Conservation Fund, the NS Natural history Museum and any other interested groups.

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. At present, Mr. Trant is approximately one month away from having a final draft of his thesis. After the defense, we will finalise the preparation of this research in the format to submit to peer-reviewed journals for consideration for publication. We anticipate three papers: the first one on the pollination ecology of *Sabatia kennedyana* (almost complete), the second one

on the genetic structure and breeding system of *Sabatia kennedyana* (in progress: the results of the genetics work will be described in next year's report) and thirdly a smaller publication on the findings resulting from our seed bank work (near completion). In addition to preparing these manuscripts for publication, we will also provide a summary of the images and results to TREPA. It is our opinion that this portion of the research initiative is very important. Without proper dialogue and dissemination of information between landowners, the public and interested organizations, the momentum and knowledge acquired is lost. We have on-going projects and students involved in this work and we will continue to work towards fostering these links.

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. The main task before us now is to finish the fitness and germination studies with SAKE, continue to disseminate the results of the SAKE research and then undertake a greater research focus with CORE over the next two years. In the summer of 2005, we continued our work with SAKE focusing on the relationship between US and Canadian populations and began examining the relative importance of clonal versus sexual reproduction in this species. In addition, we have also initiated a stronger research focus on *Coreopsis rosea*, as outlined in our 2005 grant application to your office. This is proving to be very interesting and we will present a report on these findings in the next year to the N.S. Habitat Conservation Fund.