94-06

GENETIC VARIATION AND REPRODUCTIVE ECOLOGY OF *RHODODENDRON MACROPHYLLUM* (PACIFIC RHODODENDRON) IN THE SKAGIT VALLEY AND MANNING PROVINCIAL PARK

Phase I of a Two-Year Project

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SKAGIT ENVIRONMENTAL ENDOWMENT COMMISSION

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OVERVIEW OF PROJECT

This project was the first phase of a two-year study assessing the status of the Pacific Rhododendron, *Rhododendron macrophyllum*, at its northern range limit in the upper Skagit River drainage in British Columbia. *Rhododendron macrophyllum* is rare in British Columbia, occurring only in the Skagit Valley area and nearby parts of Manning Provincial Park, and in two very small populations on Vancouver Island. It is rare or absent from the North Cascades of Washington, and the nearest Washington populations of *R. macrophyllum* are on the Olympic Peninsula and in the Cascade Mountains of southern Washington.

This attractive, showy evergreen shrub is one of the most conspicuous and distinctive plant elements in the Skagit region. Information has previously been compiled on the distribution and abundance of rhododendrons in the Skagit Valley, and on stand characteristics and associated species for most of the larger rhododendron populations (Brayshaw, 170; Ogilvie and Ceska, 1988; Desrosiers, 1994). However, genetic characteristics of these populations have not been investigated.

The overall focus of this two-year study is to examine genetic variability and reproductive output of Skagit Valley *Rhododendron macrophyllum* populations, and on the basis of this information, to evaluate (1) the genetic distinctiveness of these populations, and (2) their viability and vigour in comparison with other populations in B.C. and Washington. This information will be useful in determining appropriate management strategies and conservation measures for these plants.

This project is being carried out by Shane Ford, a graduate student in Biology at the University of Victoria, under the direction of G. A. Allen. The completed study will form the basis of a thesis for the M.Sc. degree.

Objectives for Phase I

For the first year, we had two objectives:
(1) To establish protocols for starch gel electrophoresis of isozymes in *R. macrophyllum*, and to use these to determine amounts of genetic variability in Skagit Valley

rhododendron populations (particularly whether these populations have unusually low genetic variability).
(2) To obtain information about reproductive success of Skagit Valley rhododendron populations, as assessed from flower and fruit production of individual plants.

FIELD WORK

Location of Study Sites:

During the 1994 field season, we established four study sites in the Skagit Valley Recreation Area, two in Manning Provincial Park (see attached map), and one at Rhododendron Lake, near Nanaimo on Vancouver Island.

Plant Sampling and Field Data Collection:

Field work was begun in late June. After initial reconnaissance to survey possible study sites, the six sites in the Skagit Valley/Manning Park area were selected. Each site was visited once in June and once in late August-early September. At the June visit, individual plants were selected at each site for monitoring of reproductive characteristics, and were marked with aluminum tags. At each site, the following were collected:

(1) Leaf material from 40 individual plants in each population, for isozyme electrophoresis to determine genetic characteristics of the populations. Samples were from leaves of the current season, which had already emerged.

(2) Data on site characteristics, including slope, aspect, substrate, and associated species, and on abundance of rhododendrons at each site.

(3) Data on size (height and diameter), flowering and capsule production of marked individual plants. Field work was begun toward the end of the flowering season, when plants at most of the sites had finished flowering. However, flower counts were obtainable from faded flowers or from pedicel scars on the inflorescences. All sites were visited again in early September to collect data on capsule production for

1994. The capsules of past years also persist for one to two years, and observations on these were also made.

ISOZYME PROTOCOLS AND GENETIC ANALYSES

Lab Protocols

The leaf samples for genetic analysis were taken to the University of Victoria and stored in an ultracold freezer, to be used as needed for genetic analyses.

A survey of existing literature indicated that little or no previous electrophoresis has been conducted on evergreen rhododendrons of any species. To establish protocols for *R. macrophyllum*, we carried out a considerable amount of experimentation with different buffers and/or methods of leaf preparation. The initial Tris-HCl extraction buffer was chosen because of its extensive use in other studies, and was used to prepare a large number of the samples, but test runs showed that these samples had little or no enzyme activity with this buffer. We subsequently determined that a phosphate extraction buffer gives much better results, with sharper and more detectable enzyme banding patterns.

We also found, using nursery-grown *R. macrophyllum* plants (obtained from Thimble Farms, a nursery on Saltspring Island, B.C. that specializes in native species), that we can successfully use both current-season and one-year-old leaves for these analyses, and that the leaves (if kept cool and moist) retain their metabolic activity for several days after removal from the plant.

Genetic Variation of *Rhododendron* Populations

We have now obtained good results with clear banding patterns from *R. macrophyllum* leaves for a total of 11 different enzyme systems representing approximately 17 gene loci, and have established a set of working protocols. Initial findings suggest relatively low levels of genetic variability for the Skagit Valley and Manning Park rhododendron populations. Additional samples will be collected from these populations in 1995, along

with comparison populations from Vancouver Island, the Olympic Peninsula and the southern Washington or northern Oregon Cascades.

RHODODENDRON REPRODUCTIVE ECOLOGY

Flowering

Of the six populations in which flowering and fruiting patterns were evaluated, five showed good flowering of mature plants. The sixth (Cayuse Flats in Manning Park, the most easterly population of this study) was under a fairly dense coniferous canopy, and only a few plants flowered in 1994. Flowering and fruiting data for the other five populations are reported in Table 1. All populations contained some mature plants that did not flower in 1994. However, for those plants that did produce flowers, average numbers of flowers per plant varied from 47 to 189 in the sampled populations, and average number of inflorescences varied from 4 to 23. All populations contained at least one plant (and often several) with more than 100 flowers, and the largest plant that was sampled had over 900 flowers in 90 inflorescences.

Capsule Production

Average fruit set (percentage of flowers that yielded mature capsules) varied from 39% to 72% in the five populations. There was considerable variation among individual plants within populations; a few plants produced flowers but no capsules, while other plants produced capsules from 90% of their flowers. The largest number of mature capsules found on a single plant was 879.

Flowering of rhododendrons in many areas of the Skagit Valley appeared to be less in 1994 than in previous years, based on evidence from persistent capsules. Current-year capsules can be distinguished from capsules of past years by their colour, condition, and position on the plant, and many plants that had flowered in past years had fewer or no flower clusters in 1994. However, our results from the 1994 growing season indicate substantial capsule production in each of the sampled populations, suggesting that large amounts of seed were produced even if this was a below-average seed year.

GOALS FOR PHASE II

Field Work and Reproductive Ecology

Further plant material for isozyme electrophoresis will be collected in May and June 1995, from both Skagit Valley populations and from populations in Washington and elsewhere in B.C. to be used for comparisons. Flower production in the populations marked in 1994 will be censused at the same time. Other field work will include (1) hand-pollination experiments to determine whether cross-pollination between plants is required for seed set; and (2) survey of Skagit Valley study populations for evidence of seedling germination and establishment. Capsule production will be determined for marked plants in all populations in late August or early September.

Lab Work and Genetic Analyses

Electrophoretic analyses will be continued at the University of Victoria during the summer as field work permits. Completion of this work is expected by February 1996. Data on flowering, fruiting and seedling recruitment will also be analyzed.

LITERATURE CITED

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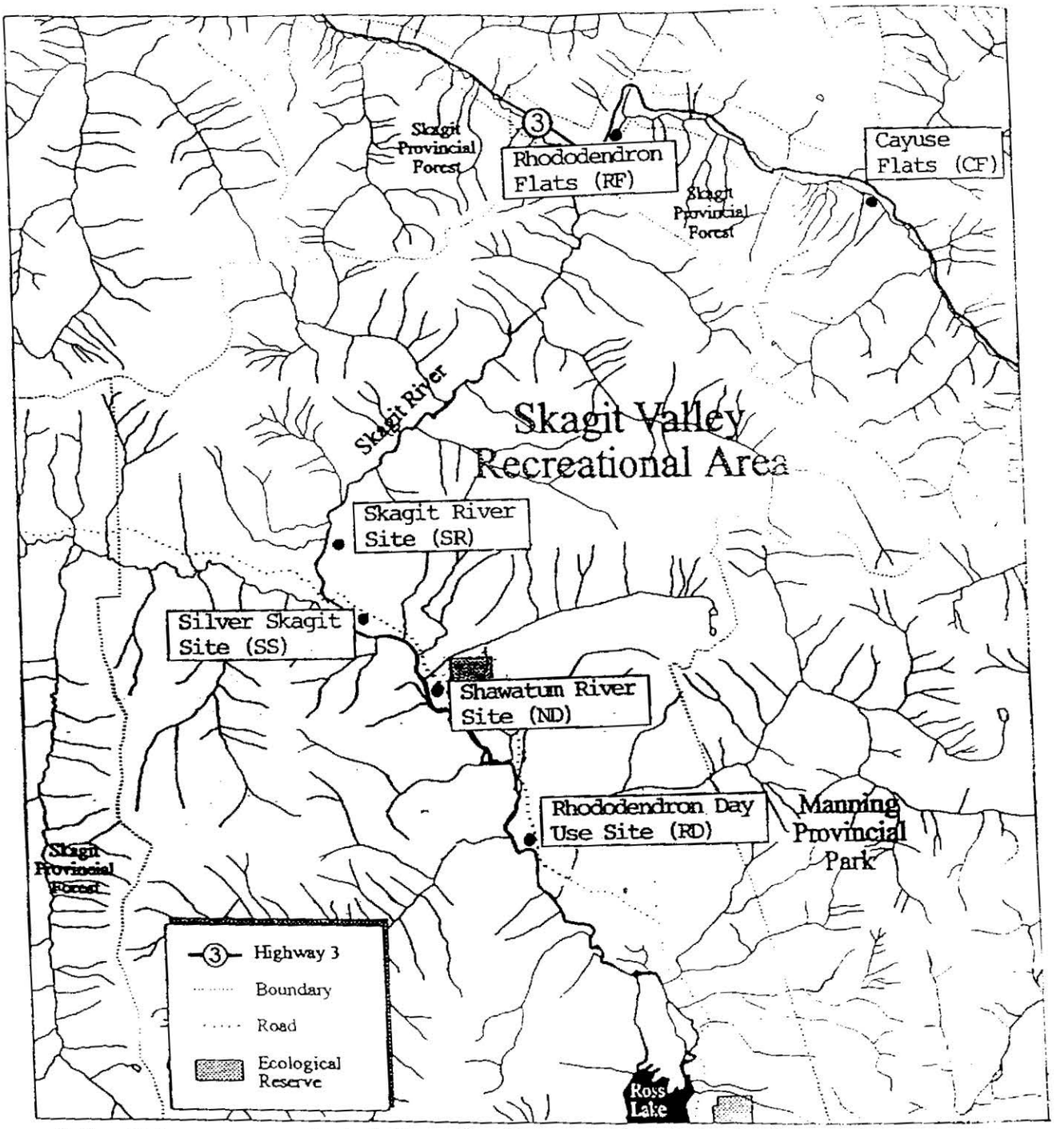
Desrosiers, J. 1994. Pacific Rhododendrons (Rhododendron macrophyllum D. Don ex G. Don) of the Skagit River Valley: A Preliminary Report. B. C. Ministry of Forests.

Ogilvie, R. T. and A. Ceska. 1988. Rare Vascular Plants of the Skagit Valley, British Columbia. Skagit Environmental Endowment Commission. Project No. 88-04. Unpublished report.

Rhododendron macrophyllum Project:
Localities of Skagit Valley Study Sites.



N.B. This map was adapted from the Conservation Data Centre status report on Rhododendron macrophyllum by Julie Desrosiers and Carmen M. Cadrin (1994).

Table 1. Skagit Valley Rhododendron Populations -- Flower and Capsule Production in 1994.

	Flower clusters	Mature capsules	Aborted capsules	Total flowers	Fruit Set	Flowers per cluster
Site 1. RF (Rhododendron Flats)						
	24	89	81	170	0.52	7.1
	8	17	53	70	0.24	8.8
	8	3	81	84	0.04	10.5
	19	66	72	138	0.48	7.3
	11	28	76	104	0.27	9.5
	31	195	49	244	0.80	7.9
	67	376	111	487	0.77	7.3
	18	29	88	117	0.25	6.5
	23	32	170	202	0.16	8.8
	6	16	29	45	0.36	7.5
	32	101	146	247	0.41	7.7
	26	56	111	167	0.34	6.4
	22	120	58	178	0.67	8.1
	10	43	40	83	0.52	8.3
	30	143	119	262	0.55	8.7
	37	273	85	358	0.76	9.7
	12	89	21	110	0.81	9.2
	27	209	35	244	0.86	9.0
	32	209	58	267	0.78	8.3
	23	176	21	197	0.89	8.6
Means	23.3	113.5	75.2	188.7	0.52	8.25
Site 2. SR (Skagit River)						
	2	11	11	22	0.50	11.0
	4	20	15	35	0.57	8.8
	5	46	2	48	0.96	9.6
	3	12	29	41	0.29	13.7
	1	9	1	10	0.90	10.0
	4	19	18	37	0.51	9.3
	1	3	4	7	0.43	7.0
	6	3	60	63	0.05	10.5
	10	54	56	110	0.49	11.0
	8	36	60	96	0.38	12.0
Means	4.4	21.3	25.6	46.9	0.51	10.28

	Flower clusters	Mature capsules	Aborted capsules	Total flowers	Fruit Set	Flowers per cluster
Site 3. SS (Silver Skagit)						
	20	64	128	192	0.33	9.6
	29	244	66	310	0.79	10.7
	3	26	4	30	0.87	10.0
	15	94	20	114	0.82	7.6
	70	312	178	490	0.64	7.0
	9	40	19	59	0.68	6.6
	5	36	6	42	0.86	8.4
	11	27	79	106	0.25	9.6
	13	25	71	96	0.26	7.4
	34	226	156	382	0.59	11.2
	43	377	65	442	0.85	10.3
	10	86	22	108	0.80	10.8
	9	84	16	100	0.84	11.1
	7	46	9	55	0.84	7.9
	14	123	10	133	0.92	9.5
	14	63	28	91	0.69	6.5
	3	16	7	23	0.70	7.7
	2	7	6	13	0.54	6.5
	38	237	100	337	0.70	8.9
	17	55	68	123	0.45	7.2
	9	22	46	68	0.32	7.6
Means	17.9	105.2	52.6	157.8	0.65	8.67
Site 4. ND (Shawatum River)						
	2	5	14	19	0.26	9.5
	1	0	4	4	0.00	4.0
	14	83	31	114	0.73	8.1
	6	26	31	57	0.46	9.5
	11	69	52	121	0.57	11.0
	4	27	11	38	0.71	9.5
	7	33	27	60	0.55	8.6
	5	22	15	37	0.59	7.4
	3	8	13	21	0.38	7.0
	10	3	109	112	0.03	11.2
	4	0	44	44	0.00	11.0
Means	6.1	25.1	31.9	57.0	0.39	8.80

	Flower clusters	Mature capsules	Aborted capsules	Total flowers	Fruit Set	Flowers per cluster
Site 5. RD (Rhododendron day use area)						
	49	382	70	452	0.85	9.2
	100	339	430	769	0.44	7.7
	5	30	5	35	0.86	7.0
	21	174	20	194	0.90	9.2
	26	145	58	203	0.71	7.8
	6	52	9	61	0.85	10.2
	5	17	8	25	0.68	5.0
	10	49	33	82	0.60	8.2
	33	272	57	329	0.83	10.0
	1	7	1	8	0.88	8.0
	16	81	50	131	0.62	8.2
	10	34	42	76	0.45	7.6
	2	6	4	10	0.60	5.0
	8	39	25	64	0.61	8.0
	3	14	6	20	0.70	6.7
	4	27	10	37	0.73	9.3
	1	6	0	6	1.00	6.0
	9	55	12	67	0.82	7.4
	90	879	88	967	0.91	10.7
	6	36	9	45	0.80	7.5
	10	35	55	90	0.39	9.0
Means	19.8	127.6	47.2	174.8	0.72	7.99