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Title AN EXPERIMENTAL TAXONOMIC STUDY OF A NORTHWEST AMERICAN POLYPLOID SPECIES, SCROPHULARIA LANCEOLATA PURSH

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A study was made of the taxonomic relationships of three subspecies of Scrophularia lanceolata Pursh native to the Pacific Northwest. Experimental methods were employed including: uniform garden studies; artificial intersubspecific hybridizations; determinations of seed and pollen viabilities of parental, hybrid and selfed plants; morphological and cytological studies; as well as ecological observations of natural populations.

Transplants and seed of subspecies californica, lanceolata and oregana, collected from ten natural populations, served as the experimental material. The taxa are all high polyploids, having a diploid number of circa 96 chromosomes.

One-hundred-twenty F_1 plants were grown from various hybridizations between the three subspecies. The F_1 hybrids displayed heterosis, but inbreeding depression was observed in the 45 S_1 and

three F_2 plants studied. Seed set and seed viability of the F_1 generation were high, averaging 98.3 percent in ten crosses. Seed set and seed viability in the S_1 and F_2 generations were lower, with viabilities averaging 81.7 percent and 51.3 percent respectively. Pollen viability of selected F_1, F_2 and S_1 plants, as measured by staining methods, was not significantly lower than in plants from natural populations.

Twenty S_1 plants, derived from self-pollination of an individual of ssp. oregana from Cape Arago, Oregon, were quite uniform in their corolla color and form. This uniformity suggests a high degree of homozygosity, which may have resulted from facultative autogamy in the natural population. This would be an advantageous breeding system for a pioneering genus like Scrophularia.

Three self-pollinated F_1 plants produced an average of 51.3 percent viable seed, while five F_1 plants that were outcrossed to members of the three native subspecies yielded 94.8 percent viable seed. The probability of natural gene exchange and introgression in regions of sympatric association of the subspecies is supported by the heterosis in the experimental F_1 generations and their high viable seed set and hybrid vigor when outcrossed or backcrossed.

Plants displaying phenotypes that simulated other disjunct taxa of Scrophularia were obtained from randomly collected, open-pollinated seed from natural populations, notwithstanding phenotypic floral uniformity in the parent natural populations. This result seemed to indicate the existence in nature of strong selection pressure and probable genetic homeostasis. Reproduction by vegetative growth (rhizomes) and autogamy also contribute to morphological uniformity in some populations.

Physiological races not marked by morphological differences may be phylogenetically more significant than morphological subspecies. A seaside population of ssp. *oregana* at Ona Beach was very similar in its floral phenotype to plants in a population of the same subspecies at approximately 3, 500 feet on Mary's Peak, although the climates at the two sites differ in many respects. Three F_1 hybrids of a mating between ssp. *lanceolata* X ssp. *californica* displayed physiological derangement. Subspecies *californica* had reduced vegetative vigor and survival under the uniform environmental conditions at Corvallis, suggesting that latitudinal races have evolved in the species complex differing perhaps in their response to photoperiodicity.

Two F_2 plants of a cross between ssp. *lanceolata* X ssp. *oregana* segregated for the taxonomic characters most often used to separate the two subspecies, that is, staminodial shape, corolla color and leaf shape. Thirty-two F_1 plants were intermediate with respect to these characters. Genetic evidence indicates complex polygenic inheritance for most of the morphological characters, although differences in leaf, staminode and color of the corolla may be due to a low number of genes.

A criticism is presented of earlier taxonomic studies in which the three taxa were considered to be separate species. On the basis of the experimental results, the lack of consistent morphological differences, and the presence of intergrading populations in natural sympatric areas, the taxa are here treated as conspecific and are made subspecies of *Scrophularia lanceolata* Pursh.

AN EXPERIMENTAL TAXONOMIC STUDY OF A NORTHWEST AMERICAN POLYPLOID SPECIES, *SCROPHULARIA LANCEOLATA* PURSH

by

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Curt Gerald Carlbom

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AN EXPERIMENTAL TAXONOMIC STUDY OF A NORTHWEST AMERICAN POLYPLOID SPECIES *SCROPHULARIA LANCEOLATA* PURSH

INTRODUCTION

The genus *Scrophularia*, commonly known as the "figworts", is a large and widespread taxon composed principally of herbaceous perennials and semi-woody shrubs. The genus, a member of the family Scrophulariaceae, is restricted primarily to the Palearctic biota of the North Temperate Zone. Over 150 species have been described. There are many inherent taxonomic difficulties within this genus and species delimitation on morphological characters alone has not provided an acceptable phylogenetic classification.

Stiefelhagen (50, p. 406-496), a German monographer, first clarified many of the problems in *Scrophularia* for the modern systematist. He considered *Scrophularia* to be one of the most species-rich genera of phanerogams native to the North Temperate Zone. Both Shaw (47, p. 147-178) and the present author have been able to corroborate many of Stiefelhagen's judgements with respect to *Scrophularia* in North America.

The present treatment is limited in its scope to an experimental study of the phylogenetic and evolutionary relationships of three taxa of *Scrophularia* which are indigenous to the Pacific Northwest. These taxa have been variously treated by other taxonomists and monographers such as Abrams (1, Vol 3, p. 770-774), Hitchcock et. al. (29, part 4, p. 411-412), Jepson (30, p. 906), Munz (42, p. 642-643), Pennell (44, p. 171-173) and Shaw (47, p. 174-177). The taxonomic problems present in these taxa are much the same as in other species of the genus; however, in addition there are a number of unique characteristics and problems inherent in the North American members. Important to these problems are the presence of high polyploidy and absence of strong genetic barriers among the taxa, considerations which have influenced the author in his final systematic treatment.

The experimental taxonomic methods that have been employed in this study include: experimental hybridizations of cultivated material grown under uniform garden conditions in the greenhouse; studies of phenotypic variation of transplants, hybrids and plants grown from randomly collected seed in natural populations; chromosome counts and cytological studies; determinations of seed and pollen viabilities; ecological studies in the field, including studies of habitat requirements; observations of floral and vegetative variation in the natural populations; phytogeographical studies; comparative morphological studies of living plants and herbarium exsiccatae; and considerations of introgressive hybridization in regions of sympatric association.

By collating the data obtained by experimental taxonomic methods, the author has endeavored to construct a classification which reflects the true evolutionary relationships of the three taxa that have been studied in this investigation.

MATERIALS AND METHODS

Transplants and Seed Collections

The initial collections of rhizomes and seed of *Scrophularia lanceolata* Pursh ssp. *oregana* (Pennell) Carlb. were made in the summer of 1961 by Dr. Kenton Chambers at Cape Arago near Coos Bay, Oregon. Additional collections, including field-dug rhizomes and mature seed, were added in the fall of 1961 by the author, Dr. Chambers and Mr. Milton L. Dean. In the spring of 1962, Dr. Chambers and the author collected rhizomes from two populations of *S. lanceolata* ssp. *californica* (Cham. & Schlect.) Carlb. in California; the first was from the west slope of Mt. Hamilton, approximately 50 miles east of San Francisco, and the second from a maritime habitat at New Brighton Beach near Santa Cruz. The collection from Mt. Hamilton represented plants recognized by Shaw (47, p. 174-176) as *S. californica* Cham & Schlect. ssp. *floribunda* (Greene) Shaw, and the collection from New Brighton Beach represented Shaw's *S. californica* ssp. *californica*. Rhizomes and seed of a distinct race of *S. lanceolata* ssp. *lanceolata* from a locality near Moscow, Idaho, were added, as well as rhizomes and seed of the same subspecies from a locality five miles south of Corvallis, Oregon. These collections, and others added later, are summarized in Table 1.

Table 1. ORIGIN OF POPULATION OF SCROPHULARIA EMPLOYED IN THE INFRASPECIFIC HYBRIDIZATION STUDIES.

TAXA AND ACCESSION NUMBERS	ORIGIN AND COLLECTOR *
ssp. lanceolata	
4	Benton County, Oregon. In thicket on tributory of Willamette River south of Corvallis.
372	Latah County, Idaho. Roadside thicket, Moscow. M. L. Dean 372
ssp. oregana	
1741	Coos County, Oregon. One-half mile south of Cape Arago Lighthouse, along roadside. K. L. Chambers 1741
MP	Benton County, Oregon. Near top of Mary's Peak, along road in forest of *Abies procera*.
7 and 8	Lincoln County, Oregon. Four miles west of Eddyville, on roadside bank in grove of *Alnus rubra*.
9	Lincoln County, Oregon. Ona Beach State Park, in sand along jetty.
10	Lincoln County, Oregon. Cape Perpetua State Park, along roadside bank in partially logged forest of *Picea sitchensis*.
11	Lincoln County, Oregon. One-half mile along Lobster Creek road from Alsea junction, on roadside bank with *Pseudotsuga menziesii*.
ssp. californica	
NBB	Santa Cruz County, California. Beachside thicket at New Brighton Beach. K. L. Chambers 2176.
2169	Santa Clara County, California. Along roadside on west slope of Mt. Hamilton, with *Quercus lobata* and *Q. kelloggii*. K. L. Chambers 2169.

* Accesssions in which no collector is given are the authors.

The major emphasis has been placed on the results of hybridization and breeding experiments and on observations of greenhouse-grown progeny. These data have been coupled with comparative morphological studies and ecological data gathered from field observations, in order to delimit the proposed subspecific taxa.

The plants employed in the initial hybridizations were transplanted from natural populations. They were dug either in the dormant state or during periods of early growth. All of the plants studied have perennial subterranean rootstocks that are easily transplanted, and in almost all cases they responded favorably to artificial culture in the greenhouse. Plants obtained in this fashion usually produced their first flowers within six to eight weeks after transplanting. The rootstocks were transferred to the greenhouse in polyethylene bags and planted in six-inch clay pots, or were stored in a cold room at four degrees Centigrade for periods of a few days to several weeks. There was no noticeable damage or loss of vigor with the latter treatment; instead, the early growth response of the plants was improved.

Besides transplanted rootstocks, open-pollinated seeds gathered in the field were sown, and the resulting plants also were employed in the hybridizations. Vigorous seedlings often flowered within 10 to 12 weeks after germination. These randomly collected seeds manifested a wide range of vigor. Some of the seedlings succumbed at an early stage of development, while others which grew to maturity displayed, not infrequently, very unusual and deviating phenotypes from the observed population norms. Studies of the variability within the populations of the three subspecies were limited because of the physical facilities available at the time of the study.

Seedling Culture

The seedlings were cultivated in the following way. Seed was sown in vermiculite planters, and approximately two weeks after germination, when the seedlings had developed a good strong root system and true leaves, they were transplanted into two-inch asphalt plant bands containing vermiculite. After additional growth, the seedlings were transplanted permanently into six-inch clay pots containing a soil mixture of equal parts peat moss, sand and silt-loam. The soil mixture was sterilized with steam and upon cooling, a complete, 6-12-4, inorganic fertilizer was thoroughly mixed into the soil mixture. In addition, the seedlings and the transplanted rhizomes received a supplement of organic fish fertilizer every three weeks during the growing season. The surviving seedlings reached flowering within 12 weeks after germination. These seedling plants and other transplants were then selected and used in the hybridization experiments.

Care was employed throughout the study so that each plant received the same treatment with regard to soil mixture, fertilizer, cold treatment, light, day and night temperatures. The objective was to obtain plants that displayed phenotypic variations that were a true expression of the genotypic response to the uniform environment.

Hybridization procedures

Initially in the study, individual flowers were emasculated, but after it was observed that no selfing occurred naturally in the greenhouse, this procedure was discontinued. The pollinations were artificially made in the following manner. From the plant which was to serve as the male parent, a flower was selected which had plump anthers that were just ready to dehisce. This flower was removed from the inflorescence and transferred to the stigma of the flower which was to serve as the maternal parent. The anther was brushed gently against the stigma after being teased open with a pin, until a surplus of pollen was observed on the stigmatic surface. The pollinated flower was then covered with a cellophane envelop in order to preclude the introduction of foreign pollen and to catch the seeds as they were dispersed from the ripened capsules at maturity. Crosses were made reciprocally whenever possible.

A record of the hybridizations was kept in a field note book. This included the specific maternal and paternal parents used, the date of pollination and the date of seed harvest. On the pedicel of the pollinated flower, a label was attached indicating the male parent's accession number and the date and time of pollination.

Seed Viability Determinations

Since it was not feasible to grow all the seeds obtained from the collections made in the natural populations and from the hybridizations, an evaluation of their viabilities based on germinability was substituted. Visual counts and measurements, along with dissections of randomly selected seeds were made. Tentative determinations of the percentages of aborted seeds could be obtained by visual means and by dissection, if a seed was suspected of having an aborted embryo. Final confirmation was obtained by germination trials. Viability was found to vary greatly within seed samples collected in panmictic wild populations.

The actual determination of seed germinability was carried out in the following way. Seeds were counted and placed on filter paper in sterilized Petri dishes. Distilled water was added and these seeds were permitted to imbibe water in the presence of light for one day. Next one-half of the seed sample was placed in total darkness and the other half in natural daylight. There was no difference in the germination percentages in darkness or daylight. Some seed that were germinated immediately after harvesting provided good evidence that there is not an after-ripening period in this species. However the presence of a chemical inhibitor in the seed coats of many of the genotypes was suspected, because germination was often delayed even though the seed coat was soft and could be easily penetrated by the radicle. If, on the other hand, the embryos were dissected out of the testa, they germinated rapidly in all cases.

Determination of Pollen Viability

The determination of viable pollen was accomplished by collecting young flowers from selected plants in the greenhouse and transferring them to the laboratory, where the pollen from individual flowers was dispersed on a micro-slide in a drop of dye consisting of anilin blue dissolved in equal parts of phenol, glycerine, and water. A cover slip was added, and the slide was observed under a microscope at a magnification of X450.

Pollen that displayed a well-stained and differentiated cytoplasm was judged to be viable, while inviable pollen exhibited no cytoplasm or cytoplasm which was visibly much shrunken. Viable pollen was usually large and turgid, while inviable pollen was smaller and usually somewhat collapsed.

EXPERIMENTAL RESULTS

The data obtained from the experimental studies have been drawn upon heavily in the final taxonomic treatment. Much of the experimental information has been obtained from the parental plants and the F_1 and F_2 generations of the first artificial hybridization, which was made in December 1961 between subspecies lanceolata (accession number 4, Corvallis) and subspecies oregana (accession number 1741, Cape Arago). A total of 32 F_1 and three F_2 plants from this cross have been grown and studied. Subsequent hybridizations employing some of the above F_1 plants have been made, and other plants, representing subspecies lanceolata, oregana, and californica from localities in Idaho, Oregon, and California, have been crossed in ten combinations. Figure 10 is a graphic presentation of the intersubspecific hybridizations that were artificially made. Figure 11 shows the geographic distribution of the parental plants, the exact localities for each accession being given in Table I. In Table II are presented the percentages of viable seed which were obtained from the different crosses. As can be seen from Table II, there are no, or perhaps only weak genetic barriers between the three subspecies in the establishment of the F' generation.

One-hundred-twenty F_1 plants, representing ten different hybrid combinations, were grown to maturity. With the exception of three

F_1 plants of a mating between ssp. *lanceolata* (accession number 4, Corvallis) X ssp. *californica* (accession number NBB-2, New Brighton Beach), a pronounced heterotic response was observed in all the F_1 hybrids grown. In the exceptional cases the plants displayed physiological weakness, remained dwarfed and displayed floral derangement upon blooming. The hybrid vigor of the majority of F_1 plants is typified by the center plant in Figure 5. This heterotic effect was not unexpected in the *Scrophularia lanceolata* complex and may have been due to a combination of high polyploidy and probable facultative autogamy. While hybrid vigor was conspicuous in the F_1 hybrids, plants of S_1 and F_2 generations were less vigorous in their growth. There was lower germinability, higher embryo abortion, higher mortality of seedling plants, lagging vegetative development and attendant delay in flowering in the progeny resulting from self-pollinations as compared to those from crosses between different genotypes. The S_1 and F_2 plants flowered about 10-14 days later than F_1 plants that had been germinated at the same time. In addition to the decreased vigor of the S_1 and F_2 plants, there was a reduction in the formation of viable seed upon selfing. In three self-pollinated F_1 plants, the percentage of viable seed obtained was only 51.3 percent (Table III), and in five S_1 plants representing two of the three subspecies, the average seed set was only 81.7 percent (Table IV). These values may be compared with seed production

Table II. SEED VIABILITY FOLLOWING INTERSUBSPECIFIC AND INTRASPECIFIC HYBRIDIZATION IN SCHROPHULARIA LANCEOLATA PURSH.

Accession numbers of parents (seed parent listed first)	Number of capsules	Viable seed	Aborted seed	Average Viability
ssp. oregana X ssp. californica				
MP-1 X 2169	1	50	0	100
MP-1A X NBB-1A	2	187	15	92.6
ssp. oregana X ssp. oregana				
MP-2 X 7-10	1	49	0	100
ssp. lanceolata X ssp. californica				
372-2 X NBB-1A	2	111	6	94.8
ssp. lanceolata X ssp. oregana				
372 X 11	1	52	0	100
4-10 X MP-2	4	412	13	96.9
4 X 1741	1	175	0	100
ssp. californica X ssp. californica				
2169 X NBB-1A	1	186	1	99.5
ssp. californica X ssp. lanceolata				
2169 X 4-10	2	142	0	100
ssp. californica X ssp. oregana				
2169 X MP-2	1	181	0	100

Mean =98..3

Standard deviation = ± 2.59

Table III. SEED VIABILITY IN THE F_1 GENERATION OF SUBSPECIES LANCEOLATA (4-CORVALLIS) X SUBSPECIES OREGANA (1741-CAPE ARAGO) UPON SELFING.

Accession Numbers of F_1 Plants	Number of capsules	Viable seed	Aborted seed	Average viability
2	1	36	38	48.7
3	4	122	138	47.8
16	1	46	34	57.5

Mean = 51.33
Standard deviation = ± 5.36

from intra-and intersubspecific crosses, in which average seed viability was 98.3 percent (Table II). Other crosses which were made between different genotypes, in which all three subspecies, californica, oregana, and lanceolata, were hybridized with F_1 hybrid plants from the cross of ssp. lanceolata (accession number 4, Corvallis) X ssp. oregana (accession number 1741, Cape Arago) (Table V) also gave significant increases in seed viability. The difference between mean values for seed viability in Table III and Table V is significant at the one percent level of probability (the calculated t value with 6df = 0.63 **).

The reduced seed viabilities upon selfing and the inbreeding depression displayed by the S_1 and F_2 plants were probably the result of deleterious gene recombinations. It would be probable, for example, that some of the F_2 seeds (Table III) and S_1 seeds

(Table IV) contained homozygous recessive lethals or other incompatible gene combinations, which brought about the early embryo abortion. This suggestion was supported to some extent by determinations of pollen viabilities in the F_1 and S_1 generations. Pollen viabilities are presented in Table VI for selected hybrids and parental types. The viability of pollen was high in all of the flowers examined, but was highest in the parental plants of ssp. lanceolata.

Table IV. SEED VIABILITY OF SELECTED REPRESENTATIVE PLANTS OF SUBSPECIES CALIFORNICA AND SUBSPECIES OREGANA UPON SELFING.

Accession numbers of plants selfed	Number of capsules	Viable seed	Aborted seed	Average viability
ssp. californica				
NBB-1A	3	123	89	58.0
NBB-2	1	84	8	91.3
ssp. oregana				
MP-2	4	384	79	82.9
1741-6	1	75	20	79.0
1741-2	4	396	10	97.5

Mean = 81.71
Standard deviation = ± 13.6

The data on genetic intercompatibility support the conclusion that all three taxa studied are members of one species complex. The artificial crosses succeeded in every case, and the F_1 seed set

Table V. SEED VIABILITY FROM HYBRIDIZATIONS OF F_1 PLANTS OF THE CROSS, SSP. LANCEOLATA (CORVALLIS) X SSP. OREGANA (CAPE ARAGO), WITH PLANTS REPRESENTING SUBSPECIES LANCEOLATA, CALIFORNICA, AND OREGANA.

Accession numbers of parents	Number of capsules	Viable seed	Aborted seed	Average Viability
(4 X 1741)-8 X NBB-2 (ssp. californica)	3	193	36	84. 3
(4 X 1741)-3 X 372-2 (ssp. lanceolata)	1	78	4	95. 1
(4 X 1741)-2 X 4-6 (ssp. lanceolata)	2	312	6	98. 1
(1741-B S')-9 (ssp. oregana) X (4 X 1741)-2	3	303	10	96. 8
MP-1 (ssp. oregana) X (4 X 1741)-1	2	55	0	100

Mean = 94. 86
Standard Deviation = ± 6. 08

was high. The F_1 plants grown were mostly intermediate between their parents in vegetative and floral characters. Flowers are shown in Figure 1 of two F_1 hybrids of the cross, ssp. lanceolata X ssp. oregana. These hybrid flowers are somewhat intermediate, particularly with respect to corolla color and the size and form of the sterile stamen (staminodium). Figure 2 illustrates the normal healthy growth response displayed by the F_1 plants of this cross.

In Figure 3 individual flowers of ssp. lanceolata and ssp. oregana

Table VI. POLLEN VIABILITY IN *SCROPHULARIA LANCEOLATA* PURSH.

Accession numbers of subspecies and hybrids	Number of pollen grains counted	Percent viable pollen
ssp. *lanceolata*		
4	500	100
4-10	500	100
ssp. *lanceolata* X ssp. *californica*		
372 X NBB 1-A, F_1	400	96
372 X NBB 1, F_1	700	87
ssp. *lanceolata* X ssp. *oregana*		
4 X 1741-3, F_1	500	98
4 X 1741-5, F_1	500	91
4 X 1741-10, F_1	500	94
4 X 1741-7, F_1	500	97
ssp. *oregana* X ssp. *californica*		
MP-1 X 2169, F_1	500	94
ssp. *californica* X ssp. *lanceolata*		
2169 X 4-10, F_1	900	96
2169 X 372-2, F_1	700	92
ssp. *lanceolata* X ssp. *oregana*		
4 X 1741-2, F_2	1000	96
4 X 1741-3, F_2	800	95

are shown. The flower of ssp. *oregana* illustrated in this figure is somewhat divergent from the more typical form shown in Figure 1. It is similar, in fact, to the disjunct species, *Scrophularia atrata*, of southern California, especially in its wide and patulous corolla; yet it originated on a transplant from a population within the normal range of ssp. *oregana*. In Figure 4, plants E and F represent additional morphological variants of ssp. *oregana*. Much variability in floral form within experimental populations of the three subspecies of *Scrophularia* has been found, particularly in open-pollinated seed gathered from wild populations and cultivated in the uniform garden. This phenomenon is not usually observed, however, in natural populations. Floral variability is suspected to occur in regions where there is panmixy and probable gene exchange between subspecies. It may also be found in newly established colonies, where competition is not great, where selection has not been operative for a longer period of time, and where there has not been extensive spread of individual genotypes by vegetative reproduction. The relative uniformity of phenotypes which is observed in older established populations is suggested to be partially due to genetic homeostasis (45, p. 178-180).

Figure 4 illustrates an example of floral variability within a single plant population. The three flowers in the upper row, A, B and C, have been selected from plants grown in the uniform garden

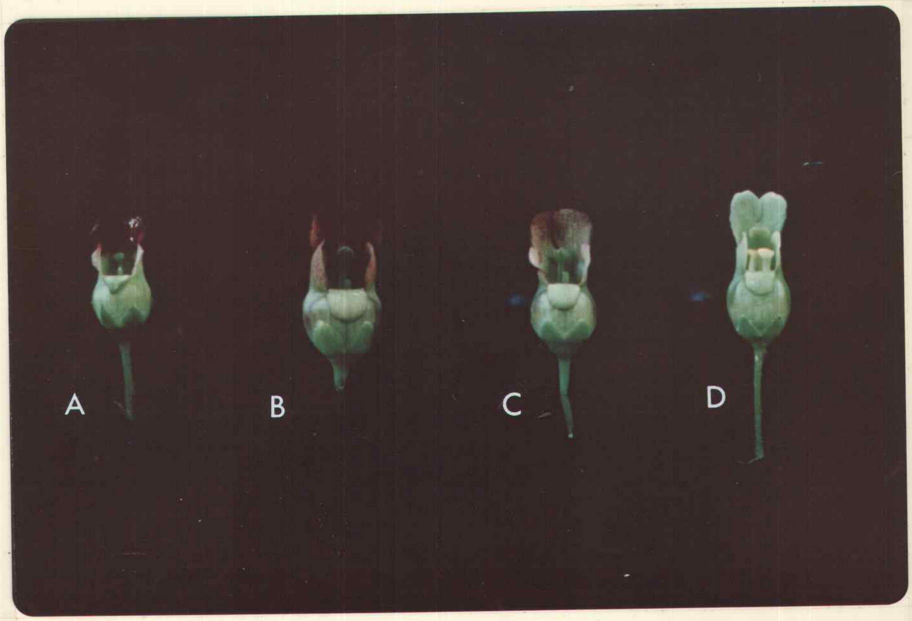

Figure 1. Flowers of subspecies <u>oregana</u> (1741-Cape Arago) (A); subspecies <u>lanceolata</u> (4-Corvallis) (D); and flowers from two of their F_1 hybrids, (B and C).

Figure 2. F_1 hybrid of subspecies <u>lanceolata</u> (4-Corvallis) X subspecies <u>oregana</u> (1741-Cape Arago).

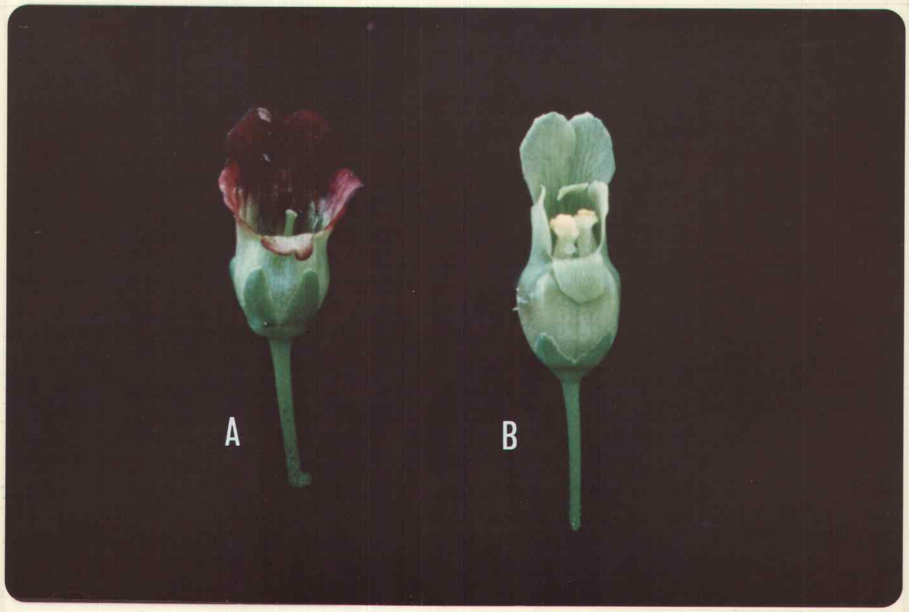

Figure 3. Flowers of subspecies oregana (TP-7 Eddyville) (A), and subspecies lanceolata (4-Corvallis) (B).

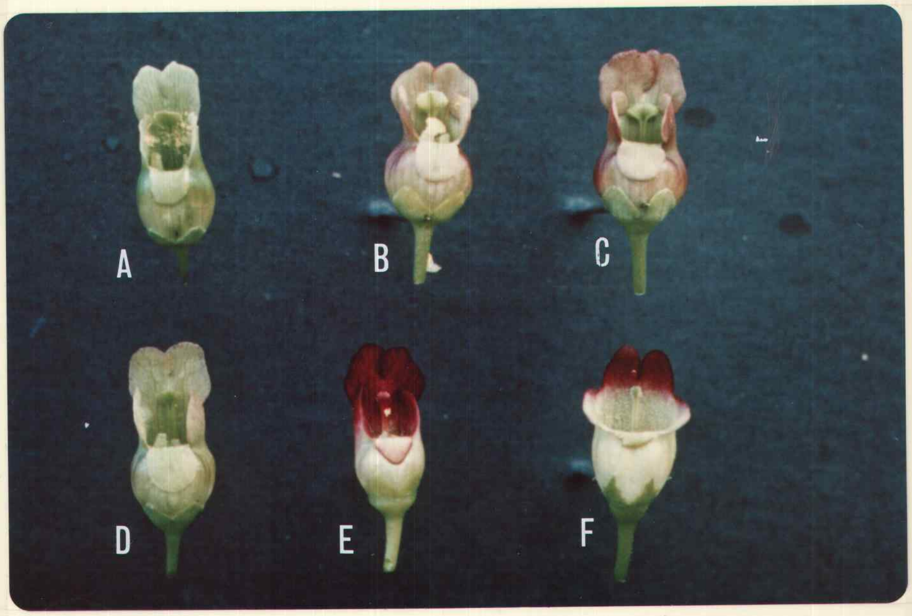

Figure 4. Floral variability in subspecies lanceolata (A, B, C, and D), and subspecies oregana (E and F).

from seed of a single open-pollinated capsule of ssp. lanceolata (accession number 4, Corvallis). Variation is apparent in the shape and pigmentation of the corollas, and in the shape of the staminodium. Flower D, in the lower row, appears to be intermediate to A and B, above. It represents a transplant (accession number 372) from Moscow, Idaho. Flowers E and F, in the lower row, are from seedling plants from two different populations of ssp. oregana (accession numbers 7 and 9) occurring approximately 30 miles apart. Figure 4 also shows distinctly the so-called "key" character differences between ssp. lanceolata and ssp. oregana, namely the broad flabellate staminodium, which is wider than long, and the generally lighter pigmentation of the corolla in ssp. lanceolata. The character of corolla color is the more variable and is undoubtedly under the control of many genes. The staminodial character may be more constant and perhaps is the result of monogenic inheritance with possible ancillary or complementary genes contributing minor variation and modification. In the case of ssp. oregana, the staminodium is longer than wide, club shaped (clavate), or sometimes more or less acutely pointed. The floral parts are mostly deep crimson, but much variation in pigment color is present and seems to be under the control of several interacting genes. The degree of pigmentation likewise is variable; in flower E, for example, anthocyanin is present in the staminodium, while the staminodium of flower F is entirely devoid of pigment.

Further studies of the genetic relationship of ssp. lanceolata and ssp. oregana involved crossing F_1 hybrid plants with themselves, with their parents, and with other genotypes from all three subspecies. Thirty-two F_1 plants were grown to maturity from 35 germinated seedlings. The three seedlings that succumbed were apparently injured during the process of transplantation. Self-pollinating the F_1 plants gave rise to seed displaying an unusually high rate of abortion and low germinability (Table III). Twenty-five viable seed from a single capsule were planted. Poor germinability and high seedling mortality eliminated all but three plants, which, however, developed normally and flowered. These three plants displayed growth depression during the early stages of vegetative development, but they did produce normal flowers and have survived for over a year in pot culture in the greenhouse. In Figure 5, the growth of two F_2 individuals is compared with that of plants of the F_1 and parental generations.

There was segregation in the F_2 plants with respect to the staminodial and leaf characteristics of the two parents. In Figures 6 and 7, the F_2 plant on the left has conspicuously lanceolate leaves typical of the ssp. lanceolata parent of the original cross. However, the staminodium of this plant was clavate like that of the other parent, ssp. oregana. The F_2 plant on the right has leaves that are very

Figure 5. Plants of subspecies lanceolata (4-Corvallis) (A); subspecies oregana (1741-Cape Arago) (E); their F_1 (C); and two F_2 plants, (B and D). (Note the conspicuous hybrid vigor of the F_1.)

Figure 6. Two F_2 hybrids of the cross, subspecies lanceolata (4-Corvallis) X subspecies oregana (1741-Cape Arago).

Figure 7. An enlarged view of leaves of the two plants shown in figure 6.

similar to ssp. oregana, but its flowers possess staminodia which are of the broadly flabellate, lanceolata type. The corolla coloration of the latter plant is paler than that of the former, suggesting that there may be linkage or pleiotropic effects of genes responsible for the inheritance of the staminodial and corolla color traits. The third F_2 plant was intermediate and much like the F_1 plants.

Pollen viability was high in the three surviving F_2 plants (Table VI), which provides additional support for the interpretation of lanceolata and oregana as subspecies of a single species.

DISCUSSION

Species Concept

It is only natural that a species concept be considered in an experimental taxonomic revision such as this one. I feel that a definition of species should embrace a genetic concept that is in league with the evolutionary development of the taxa under consideration. A species concept must not be simply arbitrary or expedient to the final classification. Since experimentation provides the foundation of this method of taxonomy, the species concept is greatly dependent on the data derived from the experiment. Muntzing, Tedin and Turesson (41, p. 9) consider the experiment to be the only safe method of distinguishing between genetical differences and mere modifications caused by environment. They are of the opinion that the experiment may alter previous taxonomic grouping, but in return it secures a deeper understanding of the biology of the plants in the widest sense. Löve and Löve (36, p. 6) acknowledge that cytotaxonomy is an effective tool for modern evolutionary classification of plants, and is the best method so far invented to study relationships and barriers between taxa at or below the species level.

A species concept must account for so many factors, it is not easy to delimit clearly the evolutionary relationships or trends in a particular group or taxon. However, a more plausible phylogeny often can be constructed from the data derived from experimental taxonomic methods. Genetic compatibility between taxa is basic to my species concept. If gene exchange can be occasioned between two plants, either naturally or artificially, and the first filial generation is highly fertile, the two plants are deemed conspecific. An essentially genetic concept of the species is the one which is most widely held by modern plant phylogenists and biologists. Examples that may be cited are Benson (7, p. 289-290), Chambers (8, p. 122), Clausen (9, 10, p. 161-178), Dobzhansky (16, p. 254-275), Harland (25, p. 104-110), Lampbrecht (33, p. 252-258), Lewis (34), Turesson (55, p. 323-324) and Winge (59). It is basically conservative with respect to the contemporary taxonomy of the phanerogams, since it leads most often to the recognition of fewer plant species. This results in a more natural regrouping of many erstwhile morphological species into single species or polytypic species complexes. Minor characters that had once served to delimit species have been placed in their proper evolutionary perspective. Furthermore, the species concept based on genetic evidence has opened a new era in taxonomy, which has led to a more thorough understanding of the evolutionary mechanisms and relationships, particularly at the intrasubgeneric level.

In the case of the Scrophularia lanceolata complex, there is extensive genetic variability and recombination of morphological features. Use of a morphological species concept leads to splitting on minor and often unreliable characters. The genetic species concept is clearly applicable in this group, however, because the members occupy the same chromosome level, and divergent morphological types are highly cross compatible. In circumscribing a large and inclusive genetic species, the taxonomist comes closer to the true biological nature of the complex.

Polyploidy

Characteristic of the American species of *Scrophularia* are polyploidy and the absence of genetic barriers between distinct taxa. *Scrophularia lanceolata* ssp. *lanceolata*, ssp. *oregana*, and ssp. *californica*, which have been studied experimentally by the present author, form a highly variable complex. Despite much genetic heterogeneity, there are few morphologically well marked taxa. Other factors, to be discussed below, contribute as well to the inconstancy of both specific and subspecific morphological distinctions.

No attempt will be made to cover the voluminous literature on polyploidy. Complete discussions can be found in Clausen, Keck and Hiesey (12, p. 1-174), Gustafsson (23), Kostoff (31), Löve and Löve (35, p. 145-163), Melchers (39), Stebbins (48, p. 298-369) and Tischler (51). Certain points should be made here, however, that pertain to polyploidy in *Scrophularia*. Gustafsson (23) regards polyploidy as favoring an increased development of the vegetative system of a plant, and this in turn may give a selective advantage to polyploid types over their diploid relatives. Gustafsson also notes that anthropochorous species, those that are favored by cultivation and other disturbances due to man, manifest a higher frequency of polyploidy. Finally, Gustafsson believes that the chromosome conditions of phanerogamic species have an important bearing on life form, growth habit and vegetative reproduction. Scrophularia lanceolata and its subspecies are characteristically anthropochorous, are polyploids, and have exploited the vegetative portion of the plant as a means of propagation and survival. Thus this species tends to corroborate Gustafsson's views.

Hagerup (24, p. 19-40), Löve and Löve (35, p. 145-163) and others have proposed that polyploid species are better adapted for more extreme environments than are diploid species. This subject is controversial, and there is not common agreement among plant taxonomists. However, the polyploid species complex of S. lanceolata seems to be an example of a taxon which is in accord with Hagerup's theory, since it is not rare to find it growing in habitats that may be considered extreme with respect to certain environmental factors. An ecotype of ssp. oregana, for example, grows on the sandy beach of the Oregon Coast, while another ecotype of the same subspecies inhabits a cold montane habitat near the top of Mary's

Peak, at 3500 feet elevation, in the Oregon Coast Range. These are examples of two rather extreme environments which are inhabited by this polyploid species. Studies by many workers, including Flovik (19, p.430-440), Löve and Löve (35, p. 157-161), and Hagerup (24 p. 30-38) have shown that the average chromosome levels of plants of higher latitudes, strand plants and desert plants tend to be higher than in species inhabiting less extreme environments.

The genetic results of polyploidy in the S. lanceolata complex consist of duplication of genomes and development of great genetic variability. Much of the variability is in the form of recessive allelic combinations which are buffered by the presence of dominant genes, but which may express themselves morphologically following self-fertilization. A reservoir of neutral and buffered gene combinations provides much of the latent variability able to be released for recombination and increased fitness in extreme environments. Polyploidy is an important evolutionary agent in the S. lanceolata complex both because of the immediate selective advantage of fit polyploid genotypes and the long term adaptability possible from gradual release of the genetic variability in its constituent genomes.

Genetic Barriers, Sympatry and Introgressive Hybridization

Genetic barriers are not strongly expressed in the North American Scrophularia. In the three subspecies considered in this study, there is morphological evidence of free gene exchange in regions where different taxa are sympatric. Individuals occur which are intermediate in both vegetative and floral characters, or which have recombinant phenotypes tending toward one or the other parent in the sympatric zone. Figure 12 shows that ssp. *oregana* and ssp. *californica* are sympatric in northern California, and ssp. *lanceolata* is sympatric with ssp. *oregana* on the western edge of the Willamette Valley and probably along the western part of the Puget Sound region in Washington.

The factors relating to natural hybridization and introgression in the *S. lanceolata* complex in northern California, Oregon and Washington are the following:

1. Artificial hybridizations can easily be made between the three taxa, ssp. *lanceolata*, ssp. *oregana* and ssp. *californica*.

2. Heterosis or hybrid vigor is present in the F_1 hybrids between plants representing the three subspecies. Vigorous hybrid F_1 plants would be able to compete favorably with both parents for survival in the sympatric zone. The hybrids would tend to have greater immediate fitness.

3. In artificially produced backcrosses of F_1 hybrids to either parent, the backcross progenies display a broad range of vigor with some of the progenies manifesting heterosis. This evidence would support natural backcrossing and the phenomenon of introgressive

hybridization between the sympatric subspecies.

4. Individual plants are found in natural populations that are intermediate between ssp. *oregana* and ssp. *lanceolata* with respect to the staminode shape. This intermediate condition has been reproduced in experimental F_1 progenies between the two subspecies. Herbarium *exsiccatae* from the sympatric regions often are morphologically intermediate for this character, as well.

5. Gene exchange may be abetted by autogamy, because intersubspecific hybrids involving inbred populations would show a gain in vigor by heterosis.

6. The disturbance of the habitat by man has allowed the expression of a wider range of phenotypic variability in the *S. lanceolata* complex.

The growth habits and genetic characteristics of *S. lanceolata* and its subspecies are important in this respect. Perenniality and pioneering tendencies (both probably favored by polyploidy), the potential for autogamous reproduction, and the lack of genetic barriers have been significant to the evolutionary success of this group.

Ecological Variation and Physiological Races

The three subspecies, *lanceolata*, *oregana* and *californica*, are all characteristically pioneering and semi-ruderal in nature. They occupy mostly disturbed habitats along roadside banks, semi-open sites along watercourses, in semi-shady thickets that are in a young stage of primary succession, among rocks, and on old dunes near the ocean. Thus they are among the early inhabitants of disturbed sites and communities that have not progressed far in succession. Although adapted to semi-shade, because of their rather large mesophytic leaves, scrophularias will survive in open sun. They respond to increased illumination by a shorter, stockier growth form and a reduction in the size of the cauline leaves. These site preferences and growth characteristics are not unique to the above three subspecies, but seem to occur in most Scrophularia species. Lagerberg (32, vol. 4, p. 1459-1460) reports that S. nodosa in Sweden inhabits places similar to the ones described above for our species. Hegi (26, vol. 6, p. 24) describes similar habitats for many central European species. It is probable that the Northwest American scrophularias have been favored in their dispersal and abundance by the advent of man and his land disturbing activities--the so-called "hybridization of the habitat" (2, p. 12-18).

I believe Stebbins (48, p. 48-49) sums up the pattern of ecological variation in S. lanceolata well when he writes, "Subspecies are based primarily on recognizable differences, while ecotypes are distinguished primarily by their reaction to the environment and may or may not possess well marked morphological differences which enable them to be recognized in the field". From the present study it would appear that the ecotypic variation based on physiological characteristics is not a tenable nor a feasible criterion for use. On the other hand, the data derived from this research tends to cast light on the biological importance of cryptic physiological traits which are not expressed morphologically in the phenotype.

Some of the evidence supporting the presence of physiological races in the *S. lanceolata* complex include the following: Subspecies *californica* displays less vegetative activity in the uniform garden at Corvallis than the two native subspecies, ssp. *oregana*, and ssp. *lanceolata*. In addition, F_1 progeny of a cross between ssp. *lanceolata* X ssp. *californica* manifested abnormal vegetative responses tending to support a differential genetic balance in the two parental genotypes. Therefore, it seems possible that the more southernly subspecies, ssp. *californica* has been selected for environmental conditions, like photoperiod, at its latitude, while the two northern subspecies, *oregana* and *lanceolata*, have adjusted to photoperiods and other conditions of the more northern latitudes.

In summary the following points can be made:

1. Morphological variation need not parallel physiological variation. The latter is more important in ecotypic adaptation.

2. The taxonomy of *Scrophularia* is not based on ecotypes. Each subspecies probably contains many ecotypes, as for example, ssp. *oregana* on the Coast versus ssp. *oregana* on Mary's Peak;

and ssp. *lanceolata* in the Willamette Valley versus the same subspecies in eastern Oregon. In Clausen, Keck and Hiesey's studies (11, vol. 1, p. 300-302), when a species like *Achillea borealis* occupied such different environments, it always showed different physiological races.

3. The genetic variability discovered in morphological characters of *Scrophularia* is no doubt also present with respect to physiological characters, although this could only be detected by means of phytotron studies or refined transplant experiments.

Factors Affecting Population Fitness

Many factors affect genetic fitness in plant groups like *Scrophularia lanceolata.* Among these are polyploidy, the absence of genetic barriers between subspecific taxa, breeding systems, and perenniality. It is worthwhile to interpret the action of these phenomena as they affect genetic variation and adaptation. Fitness might be construed as the evolutionary adjustment or adaptation that the *S. lanceolata* complex has attained through the mediation of the environment on its gene pool. Natural selection can be regarded as the result of the environmental selection process, which is the most important single agent of evolution acting on the germ plasm. Lastly, genetic homeostasis is the phenomenon of the expression of a modal phenotype in a heterozygous population. From the observations and experimental data gathered, it appears that genetic homeostasis is operative in the S. lanceolata complex.

An aspect of the breeding system that may affect the establishment of Scrophularia populations and their fitness to the environment is autogamy. From experiments reported in an earlier section it is known that plants of this genus are capable of being self-fertilized, but that the resulting seeds have a lower percentage of viability than seeds from cross-fertilization. Nonetheless, many of the selfed seed are viable, and mature plants can be raised from them. In one experiment, 20 seedlings from a self-fertilization of ssp. oregana (accession number 1741) were grown to maturity. Flowers from eight of these S_1 plants are shown in Figure 8. They are rather uniform in corolla shape and color, displaying only slight genetic variation. From the lack of conspicuous gene segregation, it would appear that the parental plant was itself not highly heterozygous, and that the natural population from which it came had undergone a period of inbreeding. Facultative autogamy of this type may have been an important mechanism for the expansive migration of S. lanceolata.

Baker (4) reports that in many closely related but disjunct pairs of species, as well as species with large gaps in their distributional ranges, autogamy is present in at least some populations. It is Baker's view that survival following long-distance dispersal is aided

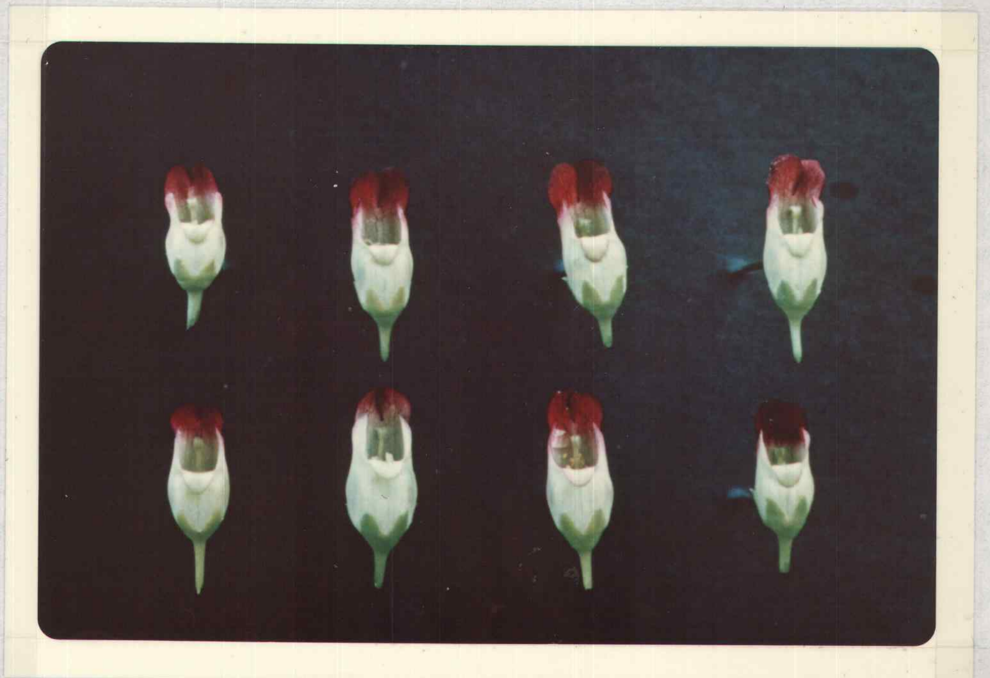

Figure 8. Flowers of eight S' plants of subspecies oregana (1741-Cape Arago).

by autogamy, since a single migrating individual is capable of establishing a population through self-fertilization. Migration is important to the survival of pioneering plants like *Scrophularia* as their population size and distribution shift rather rapidly.

From Falconer (18, p. 74-81), Wright (61, p. 232-247) and other population geneticists one gains a greater appreciation of the evolutionary processes as they probably exist in *S. lanceolata*. Wright has shown that conditions of partial differentiation into areal groups, between which a small amount of genetic interchange can occur, are the most favorable for rapid evolution. It is accordingly probable that a species inhabiting a large diversified area, and differentiated into a number of subspecies between which reasonable intercrossing occurs, will continue to evolve as a polytypic species without the subspecies differentiating into full species. The partial morphological discontinuity which appears to be maintained between the subspecies of *S. lanceolata* may be based on the following: regional ecotypic adaptation of populations, followed by stabilization of harmonious gene-complexes in relation to the principal adaptations, and by the consequent extension of the range of the populations possessing such gene-complexes. Where differently adapted races meet, genetic and morphological variability may increase over a narrow zone of intergradation. However, this need not result in a swamping out of the differentiated races or subspecies.

We do not yet have evidence as to whether the morphological features that distinguish the subspecies (such as flower color, staminode shape, pubescence, leaf shape, etc.) are themselves adaptive or are linked to adaptive physiological traits. Nor do we know the extent to which some of these characters may vary clinally rather than discontinuously. The general distribution pattern of this group, however, is one of allopatric, morphological subspecies that replace one another either latitudinally or longitudinally in the Northwest (Figure 12). In view of the environmental diversity, especially in climate, over this part of North America, it is likely that this morphological pattern is dependent in part on the distribution of physiological characteristics important to the adaptation and survival of the different subspecies.

TAXONOMY

Introduction

The taxa to be recognized as a result of this study are *Scrophularia lanceolata* Pursh ssp. *lanceolata*, *S. lanceolata* ssp. *oregana* (Pennell) Carlb., and *S. lanceolata* ssp. *californica* (Cham. and Schlecht.) Carlb. The taxa classified by Shaw (47, p. 174-176) as *S. californica* Cham. and Schlecht. and *S. oregana* Pennell are thus reduced to the rank of subspecies of *S. lanceolata*.

Variation and Distribution of the Subspecies

All the subspecific taxa treated below are closely related and as far as is known have no genetic barriers to hybridization. First and second generation hybrids are fully fertile, and in all of the taxa studied there is marked genetic variability. *Scrophularia lanceolata* Pursh is the earliest legitimate name that applies to this group of taxa. The most distinctive of the three subspecies is ssp. *lanceolata*, which has a conspicuous flabellate staminodium and usually lanceolate leaves. It is also the most widely distributed, extending throughout the northern and central parts of the United States, from the Atlantic Coast to Puget Sound, and south through the Willamette Valley and the northeastern and north central parts of California. The taxon, ssp. *oregana*, occurs along the northern coast of California, in western Oregon, from the coast to the western edge of the Willamette Valley (where it is sympatric with ssp. lanceolata), and in western Washington and British Columbia, especially in regions under maritime influence. Subspecies oregana and ssp. californica are more similar to each other than either is to ssp. lanceolata. Both are characterized by ovate or ovate-lanceolate leaves and a clavate of filiform staminodium. Shaw (47, p. 175) who gave these taxa species rank, distinguishes them primarily on corolla color, describing S. californica as, "Corolla light maroon or garnet brown; inflorescence narrow and compact to wide and spreading. West of Sierra Nevada, California," and S. oregana as, "Corolla dark maroon, inflorescence narrow. Coast of Oregon and Washington."

The discussion of ssp. californica by Shaw illustrates very well the difficulties that are presented by the ecotypic variation in this taxon. He states, "This is the most confusing and difficult western North American species. It is extremely variable and widespread, occurring all along the coast of California and on both sides of the Great Valley of California. It is found in a variety of habitats, especially on roadside cuts and in the chaparral, from sea level to 3,300 feet elevation. ... The variability is most striking in the nature of the inflorescence, color of the corolla, and the margin of the leaf. Of these three kinds of variability, only leaf margin seems to be correlated with geographical distribution. The treatment herein included is based upon morphological and distributional studies of herbarium and field observations." Shaw distinguished in his study two entities which he treated as subspecies of *S. californica.*

Comparison with Previous Taxonomic Treatments

My decision to recognize the taxa *oregana* and *californica* as subspecies of *Scrophularia lanceolata* is at variance with the taxonomic treatments of these entities by Francis W. Pennell and Richard J. Shaw. The difference is due in part to the emphasis I have placed on genetic compatibility as a species criterion, and in part to my opinion that the morphological distinctions are too minor and inconstant to support, in the face of high crossability, the recognition of three or more distinct species. Pennell and Shaw employed principally morphological characters in defining species of *Scrophularia.* According to Shaw (47, p. 177); with regard to *S. oregana*, "In 1928, Pennell was emphatic about the distinctness of this taxon from *S. californica*, but in 1947, he changed his opinion and considered *S. californica* as ranging from Vancouver Island to southern California. After studying *S. oregana* in the field and growing it for two years in the greenhouse, I am convinced that morphologically and geographically it is worthy of specific rank. The large, open throat of the blackish-maroon corolla, the narrow, ascending inflorescence, and the tendency for the corollas to persist on developing fruits are characteristics which readily set S. oregana apart from S. californica." In another place, Shaw (47, p. 166) explains his use of the criterion of geographic range in separating these species. He states, "In the present treatment the natural units of Scrophularia have been delimited by the combined information obtained from morphology, cytology, hybridization and geographic distribution. A criterion of strict genetic isolation can not be used in treating the Nearctic species of Scrophularia. If such an approach were used, it would result in the "lumping" of most or all of the species into one. Geographic barriers seem more important in isolating the western North American species." I do not agree with Shaw's use of geographical isolation in this instance. These taxa of Scrophularia replace one another geographically through zones of intergradation, as would be expected of interfertile subspecies (see Figure 12). I can find no consistent characteristics--morphological, genetic, or geographical--by which to delimit distinct species.

The Taxonomic Significance of Variation in Ssp. Oregana

The morphological characters which Shaw considered to delimit species are mostly quantitative characters that are present to a greater or lesser degree in both ssp. oregana and ssp. californica. The tendency for corollas to have a dark maroon coloration is a rather consistent characteristic of ssp. oregana, but it is not the *oregana.* The tendency for persistent corollas exists to a lesser degree in ssp. *californica,* as well. A trait so obviously affected by environmental as well as genetic factors is hardly to be recommended for use in taxonomy.

Floral structure is also variable in ssp. *oregana*. In my greenhouse cultures, certain individual plants simulated those of other western North American populations recognized by Shaw as distinct species. I have included some examples of this floral variability in different populations of ssp. *oregana* (see Figures 3, 4 and 8). The classical studies of *Antirrhinum* by Bauer (6, p. 295-302) help explain this phenomenon in *Scrophularia.* Bauer was able to reconstitute a number of disjunct species of *Antirrhinum* native to Italy, North Africa, Spain and Portugal by breeding experiments using a few species of *Antirrhinum* native to Spain. He recovered F_1 and F_2 progenies which were identical to other morphologically circumscribed species of *Antirrhinum.* Bauer and other workers (33, p. 228-229) have concluded that there is only a single species instead of the many that have been described, and that the several distinct morphological and geographical entities are physiological races or ecotypes of *Antirrhinum majus.* If this phenomenon can be applied to *Scrophularia,* observations of the variant flowers in ssp. *oregana* may provide additional evidence on the conspecificity of the three or more subspecies comprising the polyploid complex, *S. lanceolata.*

Leaf variation follows a similar pattern in both the subspecies and does not offer a good basis for taxonomic distinctions. On the other hand, ssp. *lanceolata* can usually be distinguished from ssp. *oregana* and ssp. *californica* by its characteristic lanceolate leaves and flabellate staminodium. Leaf size is not a valid taxonomic character, however, as it is greatly influenced by environmental conditions.

Of all the characteristics described above, the one that best marks the northern populations, grouped as ssp. *oregana* from the southern ones, grouped as ssp. *californica,* is corolla color. In ssp. *oregana* only rare exceptions to the consistently dark red or maroon corolla are found. The corollas of ssp. *californica,* as described by Shaw and as observed in cultures at Oregon State University, are light red, garnet brown, or light maroon. The distribution of flower color is not yet fully known in the areas of northern California lying between the ranges of "typical" ssp. *californica* and ssp. *oregana*. However, from available information it is unlikely that any kind of sharp discontinuity exists between these taxa. For example, despite the fact that Shaw (47, p. 175) shows only ssp. *californica* existing in the far northwest corner of California, populations with dark maroon corollas typical of ssp. *oregana* have been found approximately one mile south of Crescent City, Del Norte County, (*Carlbom 32*) and at Russian Gulch State Park, Mendocino County (K. L. Chambers, personal communication). It is not unlikely that the two subspecies grade imperceptibly into each other through much of northern California and southern Oregon.

No account has been taken here of the distinctive southern California and desert populations recognized by Shaw as *Scrophularia atrata*, *S. villosa*, and *S. desertorum*. From their chromosome numbers (see Table VII) and their general morphological relationship to ssp. *californica*, it is probable that they also are part of the *S. lanceolata* polyploid complex. They were not included in the present study, which was directed mainly toward the problem of *Scrophularia* in Oregon. The map in Figure 12, adopted from Shaw (47, p. 171-175), shows the generalized distribution patterns of the three principal subspecies of *S. lanceolata* in the Pacific States. An understanding of certain of the floral features that differentiate these three taxa may be obtained from Figure 9. Variation is most noticeable in the form and color of the corolla and the shape of the staminodium.

A Critique of the Basis for the Classification Adopted in this Study

It is difficult to employ arbitrarily defined taxonomic categories for the dynamic process of evolution, which acts upon all organisms and continually changes their morphological and genetic relationships. Nonetheless evolution is not completely fortuitious, and among related organisms there are similarities than can be distinguished.

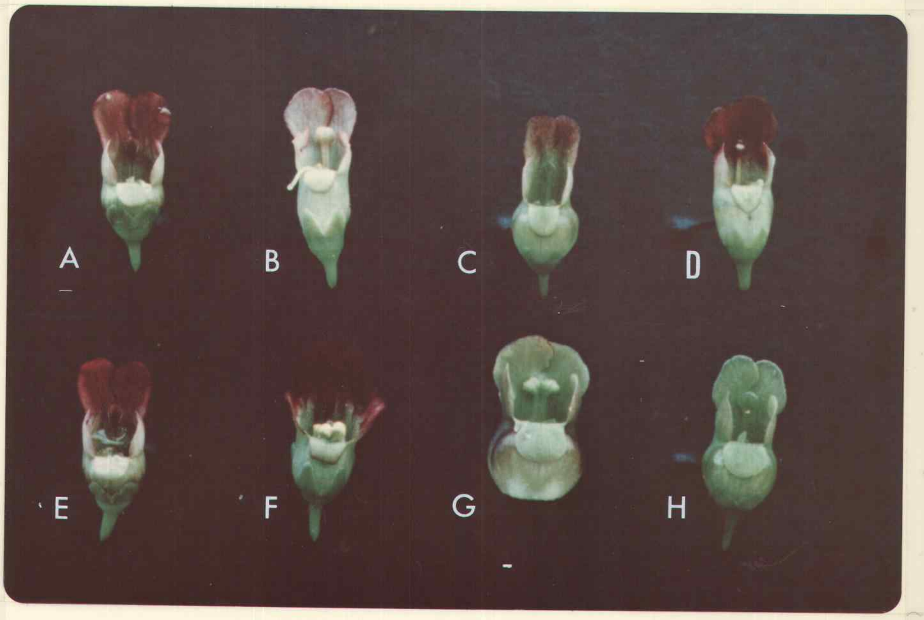

Figure 9. Flowers of subspecies californica (A, B and E), oregana (C, D and F), and lanceolata (G and H).

Although a classification may in part be arbitrary and pragmatic, it should manifest as closely as possible the evolutionary relationships of the organisms under consideration. Chambers (8, p. 122) takes the following position, "An important axiom in taxonomy is that there are many different kinds of species. Organisms exist in nature in distinct and recognizable groups, and they do so because of the nature of the biological processes of reproduction, inheritance of genetic material, and change in heredity from generation to generation (evolution). But there are so many variations in the process of reproduction and inheritance, so many modifying conditions and influencing factors, that the basic systematic categories necessarily have different significance when applied to different kinds of organisms".

On the basis of all the data and evidence that have been gathered from this study, I believe that the taxonomy proposed here reflects the dynamic and evolutionary relationships of the taxa studied, using the accepted specific and subspecific taxonomic categories.

KEY TO THE SUBSPECIES OF _SCROPHULARIA_ _LANCEOLATA_ PURSH

I. Staminodium subreniform to flabellate, broader than long; leaves mostly lanceolate (infrequently broadly ovate-lanceolate or ovate-triangular). Capsules mostly greenish-brown or brown at maturity.

... ssp. _lanceolata_

II. Staminodium clavate, subulate or obovate, mostly longer than broad; leaves mostly triangular to ovate-elliptical (rarely ovate-lanceolate). Capsules dark brown or black at maturity.

A. Corolla mostly blackish-maroon (occasionally pale maroon, reddish or yellowish-green).

. . . ssp. *oregana*

B. Corolla red or pale maroon to yellowish-brown.

. . . ssp. *californica*

SCROPHULARIA LANCEOLATA Pursh, Fl. Amer. Sept. 419, 1813.
ssp. LANCEOLATA

S. nodosa L. var. *occidentalis* Rydb. Contr. U. S. Nat. Herb. 3:517. 1896.
S. occidentalis (Rydb.) Bickn. Bull. Torrey Club 23:315. 1896.
S. nodosa L. var. *lanceolata* M. E. Jones, Contr. W. Bot. 12:67. 1908.
S. serrata Rydb. Bull. Torrey Club 36:688. 1909.
S. lanceolata Pursh var. *occidentalis* (Rydb.) Pennell, Torreya 22:82. 1922.
S. lanceolata Pursh f. *velutina* Pennell, Monog. Acad. Phila. 1:276. 1935.

Perennial herb 5-18 dm. tall, often with many clustered stems from a thickened rootstock; leaves subglabrous or with pubescence of mixed glandular and non-glandular hairs mostly on the lower surface; leaves cauline, opposite, the petiole mostly 1-5 cm. long, the blade lanceolate to ovate-lanceolate, 5-20 cm. long and 2-9 cm. wide, serrate, coarsely toothed to doubly dentate-serrate, apex acute or acuminate, base subcordate or subtruncate to rounded;

inflorescence terminal or occasionally lateral, cymose-paniculate, 10-60 cm. long and 3-15 cm. wide, conspicuously glandular, individual fascicles mostly opposite and subtended by narrowly lanceolate, inconspicuous bracts; calyx lobes 2-4 mm. long, thin, broadly rounded above to subacute, with scarious-repand margins; corolla 9-14 mm. long, yellowish-green to light maroon (rarely dark maroon) above and lighter below; staminodium 1-3 mm. wide, flabellate to subreniform, broader than long, yellowish-green; fertile stamens 4, didynamous, anthers extrorse and laterally dehiscent; 4-7 mm. long; capsule septicidally dehiscent, greenish-brown to dark brown, glabrous, 6-9 mm. long; seeds dark brown, rugose to alveolate.

Distribution: Northern California east of the Coast Range, to the western edge of the Oregon Coast Range in the Willamette Valley, west of the Olympic Mountains in the Puget Sound trough in Washington, and east to the Atlantic Coast. Rather common on disturbed soils, along roadside cuts in partial shade, thickets, watercourses.

Type: In wet meadows and woods; Pennsylvania, (not seen).

Representative specimens: IDAHO. Idaho County: Headwaters of Sheep Creek, Seven Devils Mts. M. Ownbey 2073 (UO). Latah County: Moscow, M. Dean 372 (OSC). OREGON. Baker County: Camp Creek, Cusick 2419 (UO); Section 32, dry brushy area Head 1085 (OSC); Sullivan Creek, Head 1018 (OSC); One mile above Park Creek, Head 1732 (OSC). Benton County: South of Corvallis, Sims *s. n.* (OSC); Tributary to Willamette River, 5 miles south of Corvallis, *Carlbom 4* (OSC); North of Corvallis City limits, *Carlbom 20* (OSC). Deschutes County: Gravely beach, East Lake, Paulina Mts., Deschutes National Forest, *Detling s. n.* (UO). Harney County: Wildhorse Creek, Stein Mountains, *Henderson 9155* (UO); Hillside of canyon, Frenchglen, *Hansen 857* (OSC); Along Poison Creek, 10 miles north of Burns, *Carlbom 650* (OSC). Lane County: Junction City, *Wright s. n.* (OSC). Linn County: Lost Lake, *Dean 327* (OSC); North Santiam above Detroit, *Hansen s. n.* (OSC); Nash Crater Lava Flows, *Roach s. n.* (OSC); Outside Springfield, *Parker s. n.* (OSC).

SCROPHULARIA LANCEOLATA Pursh ssp. OREGANA (Pennell) Carlb., comb. nov.

S. oregana Pennell, Bull. Torrey Club 55:316. 1928.

Perennial herb, 5-20 dm. tall, often with clustered stems from an elongate rootstock; leaves glabrescent or with glandular pubescence; leaves cauline, opposite, with petiole 1-6 cm. long, the blade ovate-elliptical to ovate-lanceolate, 5-25 cm. long and 2-12 cm. wide, the margin variable but mostly serrate, crenate or coarsely dentate, apex acute or subacuminate to obtuse, base subcordate or truncate to rounded; inflorescence terminal or occasionally lateral, cymose-paniculate, 10-70 cm. long and 3-14 cm. wide, often conspicuously glandular, bracts subtending the flowers 1-3 cm. long; calyx lobes 2-6 mm. long, variable, acute or acuminate, infrequently extending past the corolla base, margin not conspicuously scarious or repand; corolla 8-16 mm. long, mostly deep red or dark maroon above (rarely light red, garnet-brown, yellowish-brown or mostly devoid of pigmentation), paler below; staminodium 1.0-1.4 mm. wide, clavate to obovate, longer than broad, green or colored like the petals; fertile stamens 4, didynamous, anthers extrorse and laterally dehiscent; stigma capitate; style slender, 5-7 mm. long; capsule septicidally dehiscent, brownish-black to black, glabrous, 7-10 mm. long; seeds black and rugose.

Distribution: Along coasts of northern California, Oregon, Washington to British Columbia; throughout the Coast Mountains of Oregon and the Olympic Mountains of Washington at low and medium elevations, reaching to the Willamette Valley in Oregon and the Puget Sound region in Washington. Rather common on disturbed soils, especially on roadside cuts in partial shade, in thickets and along watercourses.

Type: From dry open area, surrounded by dense Sitka spruce forest, Lincoln County, Newport, Oregon, May 4-5, 1927, E. M. Harvey 57 (PH), (not seen).

Representative specimens: OREGON. Benton County: Mary's Peak, near top, Carlbom MP (OSC); Clatsop County: Along beach, Seaside, Ingram 1675 (OSC). Columbia County: Along creek bottoms, St. Helens, *Ostoj s. n.* (OSC). Coos County: Pt. Arago, *Chambers 1741* (OSC); Camp Coos Head, *Lammi s. n.* (OSC). Curry County: Sub-open, moist slopes, Winchuk River at Watermans, *Gorman s. n.* (UO). Lincoln County: Along sea coast, Yachats, *Warg s. n.* (OSC); Moist bank with *Stachys*, along Lobster Creek, *Steward 7506* (OSC); Woodland glade, Cape Perpetua, *Steward 6359* (OSC). Camp at base of Cape Perpetua, *Dean 57* (OSC); Near Toledo, *Asbahr s. n.* (OSC); Walport, *Overlander s. n.* (OSC); Four miles west of Eddyville along Yaquina River, *Carlbom 7 and 8* (OSC); Ona Beach State Park, *Carlbom 9* (OSC); Cape Perpetua State Park, *Carlbom 10* (OSC); East of Toledo, *Lawrence 3048* (OSC). WASHINGTON. Clallam County: Clallam Bay, *Jones 5966* (WTU); Neah Bay, *Thompson 9441* (WTU). Grays Harbor County: Ten miles south of Queets, *Hitchcock 19888* (WTU); 1.6 miles north of Moclips, *Jones 6542* (WTU). Jefferson County: Island at Ruby Beach, 5-6 miles north of Kalalock, *Pennell 21204* (WTU).

SCROPHULARIA LANCEOLATA Pursh, ssp. CALIFORNICA (Chamb. and Schlect.) Carlb., comb. nov.

S. californica Cham. and Schlect. Linnaea 2:585. 1827.
S. floribunda (Greene) Heller, Muhlenbergia 2:46. 1906. not Boiss. and Bal., 1856.
S. californica var *floribunda* Greene, Man. Bay Reg. Botan. 273. 1894.
S. nodosa L. var. *californica* (Cham. and Schlecht.) M. E. Jones, Contrib. W. Botan. 12:67. 1908.
S. californica var. *laciniata* Jepson, Man. Fl. Pl. Calif., 906, 1925; not *S. laciniata* Waldst. and Kit., 1805.

S. multiflora Pennell, Pro. Acad. Nat. Sci. Phila. 99:173. 1947.

S. californica ssp. floribunda (Greene) Shaw, Aliso 5:147-178. 1962.

Perennial herb, 5-16 dm. tall, with clustered stems from a shallow, elongate rootstock; leaves glabrescent or with glandular pubescence, the hairs sometimes limited to the veins and margins; leaves cauline, opposite, the petiole 1-6 cm. long, the blade ovate-elliptic to broadly ovate-lanceolate, 5-20 cm. long and 2-10 cm. wide, serrate or crenate to coarsely denate or incised, apex acute, obtuse or acuminate, base subcordate or truncate to rounded; inflorescence terminal, cymose-paniculate, 10-60 cm. long and 3-12 cm. wide, very often conspicuously glandular, uninterrupted, the bracts subtending the fascicles small and inconspicuous; calyx lobes 2-6 mm. long, acute or acuminate to obtuse and the margins narrowly scarious to slightly repand; corolla 8-16 mm. long, the dorsal petals long and narrow, garnet-brown to light crimson or maroon, ventrally paler; ventral and lateral petals variable, often recurved or deflexed; staminodium 0.7-1.2 mm. wide, clavate to obovate, longer than broad, green or colored like the petals; fertile stamens 4, didynamous, anthers extrorse and laterally dehiscent; stigma capitate; style 4-6 mm. long; capsule septicidally dehiscent, dark brown to black, glabrous, 7-10 mm long; seeds dark brown, rugose or alveolate.

Distribution: From near Crescent City, California south to about San Diego, mostly along the Pacific Coast, and in cool valleys and hills east to the Sierra Nevada Mountains. Rather common on disturbed sites, roadside cuts in partial shade, beachside thickets, along water courses.

Type: San Francisco, California, October, 1816, A. von Chamisso, (not seen).

Representative specimens: CALIFORNIA. Los Angeles County: Sepulvada Canyon, Santa Monica Mts., LeRoy Abrams 2549 (UO); Griffith Park, Los Angeles, Epling s.n. (OSC). Santa Clara County: Saratoga, Sturges 84 (OSC); West slope of Mt. Hamilton Chambers 2169 (OSC); Black Mt., Elmer 4590 (UO). Yolo County: Along creek in University of California arboretum, Davis, Miller 43 (OSC). Marin County: Moist sunny slope near Olema, Henderson s.n. (UO). San Mateo County: San Bruno, Thomas 9301 (OSC). Santa Cruz County: New Brighton Beach, beach-side thicket, Chambers 2176 (OSC).

EVOLUTIONARY DEVELOPMENT OF THE GENUS SCROPHULARIA

Evolutionarily, Scrophularia is not a young genus. Evidence for its age is seen in the great diversity of its taxa, which are physiologically adapted to the variety of climatic and edaphic conditions over its wide range in the circumboreal region. Variability in Scrophularia is the result, in part, of chromosomal evolution through allopolyploidy. Base numbers are x = 9, 10 and 13 (14, p. 314), and the chromosome numbers in certain North American species may be as high as decaploids. In the S. lanceolata complex of the Northwest, the n number is 48 (Table VII). The chromosome numbers found in most Asian and European species are somewhat lower than the majority of North American taxa, but S. canina from Europe, contains chromosomal races with the haploid numbers: 11, 13, 26, 27, 28, 29 and 56.

The only world-wide taxonomic treatment of the genus Scrophularia is the monograph by Stiefelhagen (50, p. 406-496) in 1910. This study, although based entirely on comparative morphological and phytogeographic relationships, is useful to the modern worker. Stiefelhagen's ideas concerning evolutionary relationships in the genus have been partly corroborated by more recent cytotaxonomic and experimental investigations. In his treatment, Stiefelhagen concludes that the major center of diversity and evolution of the genus is the Himalayan region of southeastern Asia. In that area are many species displaying putatively primitive characteristics, together with forms combining the morphological characteristics of the two sections of the genus. Two natural and distinct sections of *Scrophularia* are recognized by Stiefelhagen. In the section *Anastomosantes* are included herbaceous species (or species that have become secondarily woody through herbaceous ancestral forms), with well developed leaves having distinctive anastomosing venation on the abaxial side. In the section *Tomiophyllum,* Stiefelhagen includes those species that are composed mainly of xerophytic semi-shrubs or suffrutescent plants, with microphyllous leaves which lack anastomosing venation. The latter section is best represented by species native to the high plateaus of Asia Minor, Palestine and North Africa.

It may be possible to construct a plausible evolutionary scheme for the genus *Scrophularia* by employing morphological and cytotaxonomic data. It is beyond the scope of this thesis, however, to consider more than incidentally the evolutionary and phylogenetic relationships of the genus in its entirety; therefore, most of the discussion that follows has been directed to the evolutionary status of North American *Scrophularia*.

Shaw (47, p. 148) remarked that Stiefelhagen, in his revision of the genus *Scrophularia* in 1910, was less familiar with the American members of the genus than with the European species.

He treated the American plants under two species, *S. macrantha* Greene and *S. nodosa* L. He recognized *S. lanceolata* and *S. marilandica* as distinct varieties of *S. nodosa.* In recent years, *S. macrantha* was renamed *S. neomexicana* by Shaw (47, p. 170), probably as the result of an error in nomenclatural judgement.

Although Stiefelhagen believed *S. marilandica* was identical with the European species, *S. nodosa*, there is cytotaxonomic evidence that they are separate species. *Scrophularia nodosa* has an *n* number of 18 chromosomes, but *S. marilandica* and the taxa treated by Shaw as *S. lanceolata*, *S. oregana*, *S. californica*, *S. desertorum*, *S. villosa*, *S. neomexicana* (*macrantha*), and *S. atrata* have *n* numbers of circa 43-48. The other native species of North America that has been studied cytotaxonomically is *S. montana*, which has an *n* number of circa 35-38 chromosomes (Table VII.).

It is probable that *S. nodosa* or a *S. nodosa*-like species was one of the ancestral parents of the North American polyploid species complex of *Scrophularia*. It is the opinion of this writer that the North American taxa comprise a young, rapidly evolving polyploid branch of the section *Anastomosantes*. This assumption is based mainly on morphological similarities: anastomosing venation, herbaceous habit and mostly mesophytic leaves (there are some exceptions, but these appear to be the result of secondary adaptation). *Anastomosantes* was considered by Stiefelhagen to be the evolutionarily more advanced of the two sections. Chromosomal evidence would seem to support this section as being actively evolving through polyploid speciation. Most of the member taxa are tetraploids or higher polyploids based on *x* numbers of 9, 10 and 13, while the few taxa counted belonging to the section *Tomiophyllum* are diploids.

As mentioned above, all but one of the North American species have haploid numbers in the range of 45-48. Because genetic barriers are absent or only feebly expressed in all hybridizations thus far attempted between the taxa with 48-49 chromosomes, an argument can be raised to include them all in one genetic species. *Scrophularia montana*, which has *n* = 35-38, probably would not form fertile hybrids with the other taxa and should be kept as a distinct species. In summary, this author suggests the recognition of only two species of *Scrophularia* in North America, *S. lanceolata* Pursh and *S. montana* Wooten. The other taxa previously described as species could be relegated to subspecific status under *S. lanceolata* if genetic evidence showed them all to be interfertile.

Table VII. *CHROMOSOME NUMBERS IN THE GENUS *SCROPHULARIA*.

Species and Subspecies	*N* Number	Authority
Section *Anastomosantes*		
I. Old World		
S. *aquatica* L.	40	Maude 1939
S. *ebulifolia* Hoffmgg. and Link	c. 28-29	Shaw 1962
S. *grandiflora* DC.	29	Shaw 1962
S. *lateriflora* Trauvt.	20	Shaw 1962
S. *nodosa* L.	18	Scheerer 1939
S. *peregrina* L.	18	Shaw 1962
S. *umbrosa* Dum.	26	Scheerer 1939
S. *vernalis* L.	20	Håkansson 1926
II. New World		
S. *atrata* Pennell	c. 46-48	Shaw 1962
S. *desertorum* Shaw	48	Shaw 1962
S. *lanceolata* Pursh		
ssp. *californica* Carlb.	c. 45-48; 48	Shaw 1962; Carlbom 1964
ssp. *lanceolata*	c. 46-48; 48	Shaw 1962; Carlbom 1964
ssp. *oregana* Carlb.	c. 46-48; 48	Shaw 1962; Carlbom 1964
S. *marilandica* L.	c. 43	Shaw 1962
S. *montana* Wooten	c. 35-38	Shaw 1962
S. *neomexicana* Shaw	46	Shaw 1962
S. *villosa* Pennell	c. 47-48	Shaw 1962
Section *Tomiophyllum*		
I. Old World		
S. *canina* L.	13	Rodrigues 1953
S. *lucida* L.	13	Shaw 1962
S. *variegata* Bieb.	12	Shaw 1962

* All counts from Darlington (14, p. 314) or Shaw (47. p. 157) except those of the author.

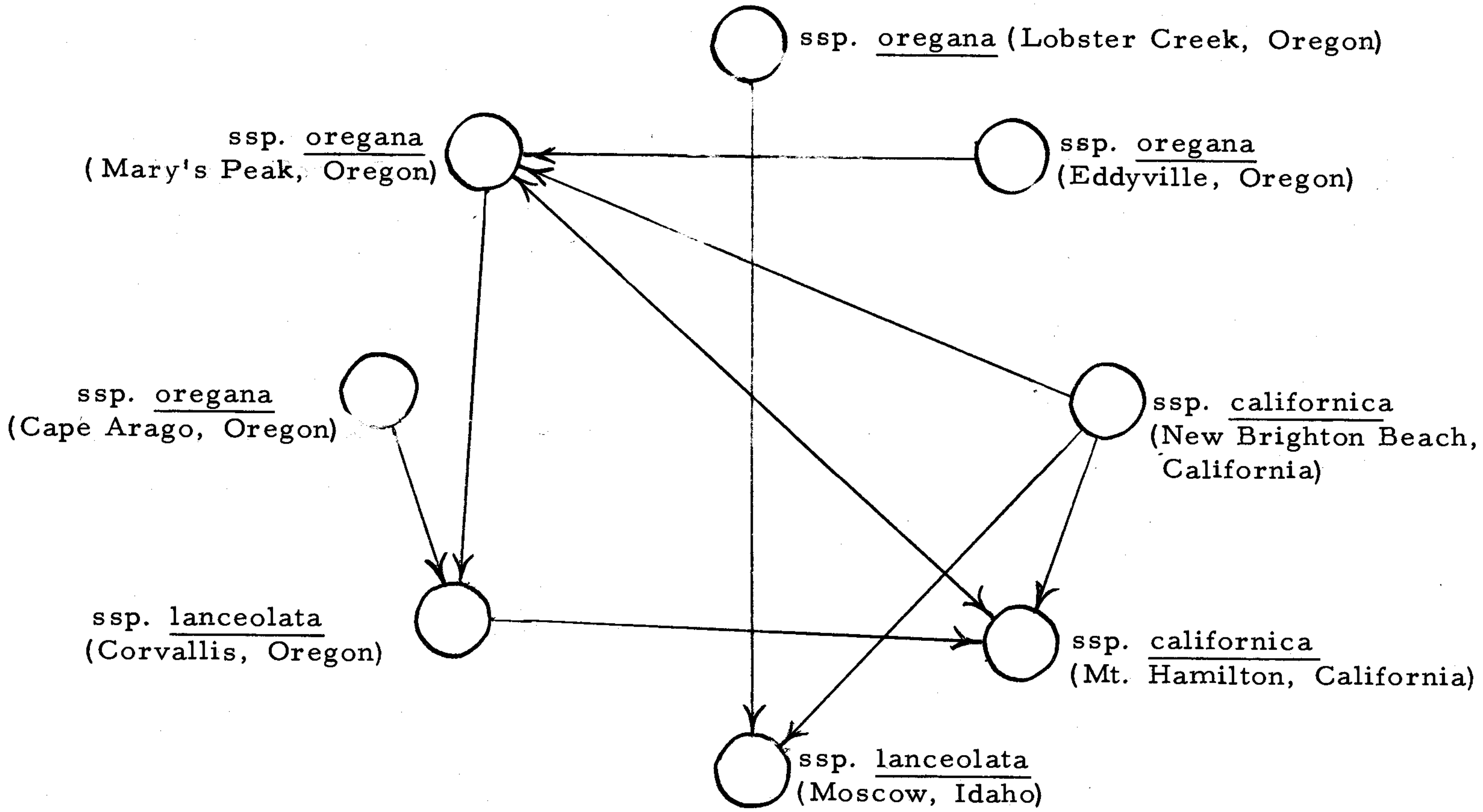

Figure 10. Intersubspecific hybridizations accomplished in Scrophularia lanceolata Pursh. (The arrows show the direction the pollen was transferred in making the hybridizations.)

Figure 11. Distributional map of populations of subspecies californica, lanceolata and oregana which were employed in the hybridization studies. (0 = ssp. californica; X = ssp. oregana; □ = ssp. lanceolata.)

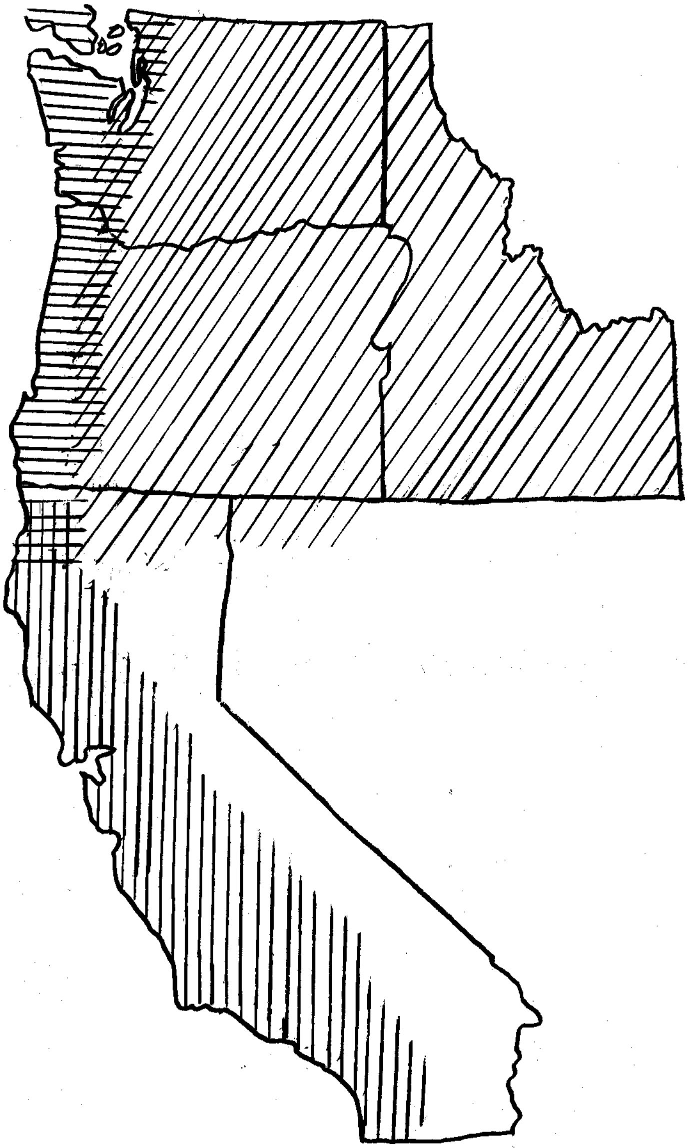

Figure 12. Distributional map of subspecies californica, lanceolata and oregana, in the Pacific states. (Note the regions of sympatric association.) (||| = ssp. californica, /// = ssp. lanceolata and ☰ = ssp. oregana.)

SUMMARY

Experimental taxonomic methods have been employed to determine the phylogenetic relationships of members of the genus Scrophularia in the Pacific Northwest. The problem was posed by the diversity of taxonomic treatments of this genus in recent floristic and monographic works. Three principal taxa are recognizable and have by some workers been considered as distinct species. As a result of the studies reported above, the author has reduced them to the level of subspecies of a single species.

Intersubspecific hybridizations between the three taxa were easily accomplished and gave rise to highly fertile F_1 progenies which displayed conspicuous heterosis. Fertility of the hybrids was measured by the percentages of viable pollen they formed and by the percentages of viable seed they produced when self-pollinated or backcrossed to the parental subspecies. Self-pollination of individuals transplanted from nature, of seedlings from randomly collected seed from wild populations, and of F_1 hybrids, produced progenies manifesting inbreeding depression and reduced seed viabilities. Individuals of the F_1 generation, when backcrossed to the parent plants or outcrossed to various genotypes representing all three subspecies, had average seed viabilities as high as their initial parents. Three F_2 plants studied from the cross of ssp. lanceolata

X ssp. oregana showed segregation for the taxonomic characters that differentiate the parental subspecies. Inheritance of morphological taxonomic traits appeared to be of a polygenic nature; however, taxonomic characters such as staminodial and leaf shapes and corolla color appeared to be under the control of fewer genes. The presence of polyploidy in all three taxa also would make the recognition of simple mendelian ratios difficult.

A self-pollinated transplant of ssp. oregana gave rise to 20 apparently rather homozygous progeny plants which were quite similar in their essential floral features. This suggests that facultative autogamy was operative in the natural population. Autogamy may have been an important factor for the successful migration in the widespread S. lanceolata polyploid complex. The fact that there was often a uniform phenotype in natural populations which displayed heterozygosity for floral and vegetative characters when cultivated in the uniform garden, indicates either that strong selection pressures eliminate most variant seedlings in nature, or that there is extensive vegetative reproduction by means of rhizomes. Facultative autogamy in some populations probably reduced the degree of heterozygosity, as well.

In regions where the subspecies are sympatric, populations containing intermediate forms occur. There was evidence of introgressive hybridization, especially with respect to corolla color and staminodial shape. The experimental hybrids between the three subspecies verify that gene exchange can occur in the regions of contact between subspecies. A high level of polyploidy is present and has abetted vegetative reproduction and the pioneering nature in the subspecies.

Physiological races seem to be more important in the successful adaptation of these taxa than are the morphological facies represented by the taxonomic subspecies. Physiological differences may occur both altitudinally and latitudinally, and in the latter case may be influenced by adaptation to photoperiod.

The three taxa studied are genetically conspecific, differ only slightly in morphology, and intergrade in nature. Therefore they have been reduced to the level of subspecies by this author, under the oldest binomial, _Scrophularia lanceolata_ Pursh.

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