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CHAPTER 7

A PROSPECTUS FOR FUTURE RESEARCH ON *VIOLA* SUBSECTION *BOREALI-AMERICANAE*

This treatment provides a foundation for a future monograph of subsection *Boreali-Americanae* that would cover in detail the distribution patterns of the taxa and the extent of hybridization among them. Such a monograph would require first extensive collecting throughout North America, including northern México. Due to the apparently broad geographic distributions of some of the taxa and the extent and frequency of hybridization and introgression among the taxa, the monographer would have to cope with an enormous sampling problem when attempting to establish accurate ranges for the orthospecies. This problem involves not only the large number of populations that would have to be sampled, but also the need for a detailed sampling in each population during both the chasmogamous and the cleistogamous phases. Cultivation of some of the sampled plants in a greenhouse may reduce the number of populations that would have to be re-sampled during the cleistogamous phase. However, it may generate another set of difficulties (such as maintenance of the plants and monitoring the release of seeds) due to the large number of plants (perhaps hundreds) that would have to be cultivated for at least one fruiting season. The second requirement for a monograph is a positive identification of each plant to discern the orthospecies. This identification can be done on the basis of the species concept and the circumscription of the taxa presented in this treatment.

There are two alternatives for obtaining positive identifications. One approach, similar to the approach practiced in this research, would require harvesting of mature seeds from each plant for SEM of the seed coat surface. The procedures for SEM and data on the orthospecies that can serve as reference points are outlined in GIL-AD (1995) and GIL-AD (in press). Extensive sampling would compensate for the relatively small sample sizes obtained in the present research, and would therefore enhance the results presented here. The major limiting factors for this approach are SEM machine time and cost.

The alternative approach for positive identifications would require harvesting of mature capsules and seeds for extraction of DNA, followed by molecular analyses of the nuclear genome (e.g., gene sequencing). Pilot studies conducted during the preliminary stages of this research on plants of subsection *Boreali-Americanae* revealed that extraction of total cellular DNA from mature capsules and seeds produces higher yields than extraction from leaves, but the amount of the DNA is relatively low, and requires amplification using PCR (Polymerase Chain Reaction) protocols. Furthermore, the DNA extracted is accompanied by secondary compounds (most likely polysaccharides) and requires further purification prior to conducting analyses. The major limiting factors for this approach are the preliminary costs and time required to locate the gene(s) that would hold promise for demonstration of ample variation between taxa at the species and subspecific levels, as well as the costs involved in subsequent extensive surveys of populations.

The present treatment represents a breakthrough in our knowledge and understanding of the of the taxa of subsection *Boreali-Americanae*. I hope that it would instigate further research on these taxa and other taxa with similar complexity.