

Hybridization and its Effect on the Systematics of Viola Subsection Boreali-Americanae

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CHAPTER 4

HYBRIDIZATION AND ITS EFFECTS ON THE SYSTEMATICS OF *VIOLA* SUBSECTION *BOREALI-AMERICANAE*

4.1. Research on Hybridization in *Viola* Subsection *Boreali-Americanae*: A Historical Perspective

The widespread and frequent natural hybridization in *Viola* is widely known (BRAINERD, 1924; GERSHOY, 1934; RUSSELL, 1954; CLAUSEN, 1951; VALENTINE, 1962). BRAINERD (1906a) noted that the tendency to interbreed has been detected among European species of *Viola* before he focused attention on hybridization in *Viola* in North America and particularly on hybridization among the taxa of subsection *Boreali-Americanae*.

Hybrids of taxa belonging to subsection *Boreali-Americanae* were initially reported by BRAINERD (1904b, 1906b, 1910a, 1912, 1913a, 1921), DOWELL (1910), and HOUSE (1905, 1924). Brainerd studied hybrids of *Viola* in detail in the wild and in cultivation. He summarized his experiments and observations in a series of papers (1906a, 1907a, 1907c, 1910a, 1924), and described and figured 82 natural hybrids (1924). Thirty four of these hybrids were the products of hybridization between taxa that are recognized as orthospecies in this study, nine involved taxa of other subsections, and the remaining were the products of hybridization between orthospecies and taxa that are recognized in this study as of putative hybrid origin.

BRAINERD (1906a) specifically noted that any two *Boreali-Americanae* species that have been growing together for several years are likely to produce hybrids, and hence the observed variability in natural populations. Early in his research (BRAINERD, 1910a) he postulated that hybridization does not add new characters to a group of species, but simply recombines characters that already exist. Yet, he was the first to observe later that natural hybridization among species in the group produces an extraordinary range of blade shapes, many of which are quite different from any found in the parental species. BRAINERD (1924) demonstrated in a number of cases segregation of blade-shape, pubescence (especially in crosses between species possessing divided blades and species possessing undivided blades), and color of capsules and seeds in the F_2 and F_3 generations, and used Mendelian laws to interpret his results. Detailed studies of crosses, such as a cross between *Viola pedatifida* and *V. sororia*, demonstrated that although the principal character differences between the species give simple segregations, and many of the F_2 and F_3 individuals show reversion to one or the other of the parental species, a substantial proportion of these offspring are considerably modified from the condition found either in the parents or in the F_1 individuals, and some of these modified types would eventually breed true (BRAINERD, 1924; STEBBINS, 1950). From his observations of the freedom with which the species cross under natural conditions, he concluded that the limit of hybridization in the genus is set only by the present lack of sympatry of different species (BRAINERD, 1907c, 1921).

GERSHOY (1928) produced experimentally 47 different hybrids between 14 taxa of subsection *Boreali-Americanae*, (including taxa that are recognized as putative hybrids in this study), and 26 reciprocal hybrids, noted their fertility, and determined their common chromosome number ($2n = 54$). GERSHOY (1934) produced additional hybrids, and listed 34 interspecific hybrids of taxa of the subsection. Nearly all the F_1 progeny were vigorous, and most were fertile. His studies did not include analysis of segregation in later generations. On the basis of both Brainerd's and Gershoy's findings, and his own cytological investigations in the genus, CLAUSEN (1951,

1964) characterized the species of the subsection as having weak [intrinsic] barriers to interbreeding.

Russell employed ANDERSON's (1949) hybridization index and pictorialized scatter diagrams on macromorphological measurements to analyze instances of hybridization, and demonstrate, in some cases, putative introgression between taxa of subsection *Boreali-Americanae*: *Viola papilionacea* introgressed into *V. nephrophylla* (RUSSELL, 1952), and *V. sororia* introgressed into *V. sagittata* (RUSSELL & COOPERRIDER, 1955). Later, RUSSELL (1955) provided evidence for hybridization and possible bi-directional introgression between populations of *V. cucullata* and *V. septentrionalis*. He concluded that introgression has proceeded to such an extent in both directions that it has obliterated many of the characteristics of the original species, making it difficult to reconstruct the original phenotypes (RUSSELL, 1955). A major problem he encountered was the determination of characters of the two parents in the preparation of a hybridization index. In some populations it was not possible to identify the orthospecies. Consequently, the ranges of variation assigned to each species were determined from a preliminary visual inspection of the data, and from published taxonomic descriptions (RUSSELL, 1955). Such constraints subject the results of the analyses to bias and jeopardy in analyzing instances of hybridization between the taxa of the subsection. RIESEBERG & WENDEL (1993) compiled a list of examples of introgression in plants, and evaluated Russell's 1955 study. They indicated that evidence for introgression in Russell's study was questionable, and interpretations other than introgression were not adequately ruled out. RUSSELL (1956a) used blade character states to analyze instances of hybridization between *V. pedatifida* and *V. sororia* in populations he sampled in Iowa. He concluded that the hybrid indexes demonstrated, though not conclusively, that hybrids are formed quite commonly between certain species, but the participating species show little evidence of introgression. Furthermore, he hypothesized that these results might reflect the problems of the genus.

Additional cases involving hybrid swarms of taxa belonging to subsection *Boreali-Americanae* were reported by HUBBARD (1955), CAMP (1961), RUSSELL (summarized in RUSSELL, 1965), and MCKINNEY & BLUM (1978, 1981). Introgression was also reported among the «stemless white» violets (ANDERSON, 1954; RUSSELL, 1954).

Natural hybridization between taxa of subsection *Boreali-Americanae* and taxa of sister subsections has been detected. BRAINERD (1924) reported natural hybrids between *Viola brittoniana* and *V. lanceolata*, and between *V. cucullata* and *V. primulifolia* (the second species in each of these pairs belongs to subsection *Stolonosae*). These hybrids were absolutely sterile, and could be propagated only by vegetative division.

GERSHOY (1928, 1934) made a large number of crosses to determine the range of hybridization possible within *Viola*. He found that there is apparently no uniform correlation between the chromosome number and the incidence of fertility or sterility. Studies on the rate of pollen tube growth in reciprocal cross-pollinations indicate that inter-sectional crosses are generally unsuccessful because of reciprocal inhibition of pollen tube growth. Species within each subsection are cytologically arranged in smaller subgroups of more closely related species, which are connected by hybrids, which in turn are only partially sterile. For example, crosses between taxa belonging to subsection *Boreali-Americanae* and taxa belonging to subsection *Stolonosae* (both of section *Plagiostigma sensu* Clausen) yielded sterile F₁ hybrids exhibiting hybrid vigor. A cross between *V. missouriensis* ($2n = 54$) and *V. jooi* ($2n = 24$; subsection *Adnatae*) yielded vigorous sterile F₁ hybrids, intermediate in character. By and large, hybrids between taxa of different sections are difficult to produce; many attempts to produce F₁ hybrids fail, and others result in seeds with embryos which are unable to develop, so that the F₁ is not even seen, and some result in weak or dwarf F₁ hybrids (CLAUSEN, 1951).

4.2. The Effects of Hybridization on the Systematics of *Viola* Subsection *Boreali-Americanae*

Hybridization, followed in some localities by introgression, has made it difficult for previous students of the subsection to discern the “core” species by using only the standard macromorphological characters. Consequently, the systematics of taxa prior to this study was in disarray.

As a result of the research and efforts of Ezra Brainerd and his successors, more data have been compiled on hybrids than on orthospecies of the subsection. However, those ample data are overshadowed by the lack of an effective method to distinguish between the orthospecies and hybrids and hybrid derivatives. Consequently, the data on the hybrids cannot be properly evaluated.

WAGNER (1968) pointed out the taxonomic importance of the recognition of hybrids, and emphasized that if hybrids are excluded from floras and manuals, identification becomes confusing, and the value of taxonomic treatments to advanced students and professional botanists is reduced. In taxonomic (and especially floristic) treatments of subsection *Boreali-Americanae*, the ideal situation would be to include descriptions of hybrids. However, since hybridization is apparently extensive and frequent, and frequently compounded by introgression, it is not possible to provide a complete coverage of all the instances of hybridization that occur in nature. Nonetheless, taxonomic treatments should provide guidelines and recommendations on how to detect and classify those hybrids.

4.3. The Detection of Hybrids

Early investigations in this study revealed that analyses of macromorphological characters are not sufficient to distinguish orthospecies from hybrids. Recognition that the characters of a suspected hybrid or deviant are mainly intermediate or grades between the suites of characters of two orthospecies is most often impossible. Knowledge of the taxa in the locality and a search for the parental taxa when a hybrid is suspected (advocated by MCKINNEY, 1992) as the means to discern the hybrids are helpful during the initial stages of the investigation in the field. However, the parental plants in some populations may be extinct or indistinguishable, with a superficial examination of macromorphology, from the hybrids. Consequently, we may have to deal with populations dominated by hybrids or introgressants. A procedure that was developed in this research, and described in below, allows identification of the orthospecies and the discernment of hybrids and putative introgressed taxa by combining analyses of micromorphological characters of the seed coat surface with macromorphological characters, supplemented by ecological and phyto-geographic data.

A Procedure for Discerning the Orthospecies. – The survey of the taxa was conducted by iterative examinations of macromorphology and micromorphology until sufficient evidence was gathered to support the delimitation of the orthospecies. In the first round of examinations, specimens were sorted into tentative groups. Specimens in each group shared a suite of macromorphological characters, including reproductive characters, that rendered them as putative representatives of an orthospecies. These groups included specimens that displayed little variation, as well as other specimens that displayed variation in a number of characters, but could still be assigned to a putative orthospecies. Several additional groups, each consisting of specimens putatively representing a taxon recognized by previous authors, were also examined, even though they deviated from the guideline of comprehensive coverage of characters. In the second round, seeds were sampled from the members of a group, and examined under a scanning electron microscope (GIL-AD, 1995; GIL-AD, in press). Then, the micromorphological characters of representative seeds of each specimen were examined using the SEM micrographs, evaluated, and compared

with the micromorphological characters of the other members of the group. Structures and patterns that were consistent were noted, and the specimens that possessed them were hypothesized as being putative orthospecies. In the third round, the macromorphological characters of each specimen in a group were re-examined, and correlations were made between suites of micromorphological characters and suites of macromorphological characters. Attempts were made to identify deviations in macromorphological characters on specimens whose seed coat micromorphology displayed deviations from the structures and patterns characteristic of the seed coats of the putative orthospecies. The fourth round included examination of petal trichomes with SEM. The data on the micromorphology of the petal trichomes was analyzed in the same fashion as the data from seed coat micromorphology, and then combined with the other data. Finally, distinct species were delimited. Specimens from each of the groups were designated as representative of an orthospecies when they displayed a consistent and distinct suite of micromorphological characters, or at least one unique micromorphological character that could be correlated with a consistent and distinct suite of macromorphological characters, as well as with ecological and phytogeographical data.

Furnished with knowledge about the delimitation of the orthospecies, we can examine whether any reliable macromorphological characters can provide reference points that can aid in detecting hybrids. Can we find any characters that would provide grounds for postulating hybridity when we compare a putative hybrid with the suite of characters that delimit an orthospecies?

During the chasmogamous phase, intermediate blade shapes, especially between taxa possessing undivided blades and taxa possessing divided blades, allow the detection of some hybrids in the field, and consequently allow postulation of hybridity. However, in many cases intermediate blade shapes are misleading in heterophyllous taxa. The problems become more acute if the plants are products of hybridization or introgression between two distinct homophyllous taxa possessing undivided blades whose shapes grade into one another. Nevertheless, a number of reproductive characters become available during the cleistogamous phase. Some hybrids produce seeds that exhibit extensive variability in color patterns among the seeds produced by a single plant. Other hybrids produce seeds exhibiting uniform color patterns among the seeds produced by a single plant. Those uniform color patterns may be novel and not match any color found on the seeds of orthospecies, or they may consist of conspicuous blotches or spots on a background color. The use of these patterns in discerning hybrids requires knowledge of the color pattern of the seeds of the orthospecies. These patterns are figured in GIL-AD (1995) and GIL-AD (in press). Capsules of hybrids may exhibit deviations in shape. They may also exhibit blends of colors of different degrees, especially if the hybrid resulted from hybridization of a species possessing green capsules and a species possessing capsules spotted red-purple. Other characters that may exhibit deviations from the characters of the closest orthospecies are the dimensions of the seeds and the habit of the peduncles of the cleistogamous flowers. Introgressants may not show any striking macromorphological deviation from one of the parents. Alternatively, they may show only few deviations, such as a blend of different types of trichomes on the petals, or presence or absence of trichomes on the spurred petal unlike the orthospecies. As noted above, a few reproductive characters can provide us with reference points, but their paucity, especially at the chasmogamous phase, and their complexity renders them difficult to use, especially to those who lack comprehensive knowledge of the taxa.

There are two alternative approaches for the identification of plants of the subsection that we encounter in the field. The first, the traditional way, is using keys and descriptions. We hypothesize that a given plant is an orthospecies. Any deviation from the suite of macromorphological characters listed for an orthospecies that we detect on this plant should lead us to postulate that the plant is a putative hybrid. Accordingly, we should seek additional sources of evidence to support this hypothesis. If we use this approach, we should be aware that any non-deviating plant might still be a cryptic introgressant. Therefore, micromorphological or other data (e.g., molecular data) would be critical for absolute establishment of the plant as an orthotaxon. The alternative approach would be to hypothesize that every plant that we encounter in the field is a putative hybrid, and seek evidence to falsify this hypothesis. Obtaining non-macromorphological

data is critical for using this approach. This approach was employed in the present research that yielded the present treatment.

4.4. The Detection of Introgression and Alternative Hypotheses

Ever since the term «introgressive hybridization» was coined by ANDERSON & HUBRICHT (1938), numerous studies documenting introgression have been published. RIESEBERG & WENDEL (1993) refined Anderson and Hubricht's definition of introgression, and characterized it as «the permanent incorporation of genes from one set of differentiated populations into another, i.e., the incorporation of alien alleles into a new, reproductively integrated population system.» Following an initial detection of macromorphological variants in populations of predominantly one species in numerous genera, various sources of data have been employed to analyze them, and to document instances of introgression. They included macromorphology, secondary compounds, isozymes, cytoplasmic markers, nuclear markers, ribosomal RNA genes, and RFLP markers (HEISER, 1973; RIESEBERG & BRUNSFELD, 1992; RIESEBERG & WENDEL, 1993).

The micromorphological data presented in GIL-AD (1995) and GIL-AD (in press), and correlations of those data with macromorphological data provide evidence that allows us to hypothesize that in some cases introgression has taken place among taxa of subsection *Boreali-Americanae*. Thus, evidence for introgression can also be sought from the examination of seed coats. When a blend of micromorphological structures characteristic of two distinct species is detected on the seed coat surface of the seeds of a putative hybrid, and it can be correlated with a blend of macromorphological characters, we can hypothesize that the plant is most likely a product of hybridization and not introgression. Conversely, when the seed coats of seeds of species «A» have structures predominantly characteristic of that species, and only traces of structures characteristic of species «B», we can hypothesize that the plant is a putatively introgressed plant. At the macromorphological level, this plant has predominantly the characters of species «A», and few deviating character states. In some cases, the macromorphological characters of species «B» cannot be readily detected on the introgressed plant, or perhaps cannot be detected at all. The detection of putative cases of introgression using micromorphological data has not been proposed before in the literature.

A major limitation of this evidence is the difficulty of quantifying the data. The seed coats of a putatively introgressed plant can be compared to the seed coats of a corresponding orthospecies, and an intuitive description can be applied to characterize the «imported» structures. Yet, the complexity of these structures and the different combination of suites of structures that characterize each of the orthospecies make it difficult to use common reference points. Thus, we cannot provide a quantitative measure that would represent the extent of the introgression detected, and a measure of the deviation between the character (structure) on the seed coat of an orthospecies and the homologous structure on the seed coat of the seeds of the introgressed plant.

One of the major problems with studies that document putative introgression is that the supporting evidence often has alternative explanations. These include the possibility that remnants of the ancestral population from which two species differentiated might have the appearance of hybrids, primary intergradation, mutations producing results similar to those of introgression, segregation in a polyploid species, the occurrence of hybrid swarms that are no longer in contact with the parental species, the presence of highly variable F₁ hybrids, and inbreeding and selection following hybridization in an autogamous species (GOTTLIEB, 1972; HEISER, 1973; RIESEBERG & WENDEL, 1993).

The last alternative explanation in the list presented above is of special interest for our problem since cleistogamy, a form of autogamy, occurs in subsection *Boreali-Americanae*. GRANT (1981) pointed out that in autogamous plants, introgression-like effects could result from inbreeding and selection of the inbred products following an initial event of outcrossing and hybridi-

zation. It would occur if morphological genes are commonly linked with viability genes, and the species involved differ allelically in respect to these systems of genes. Grant contended that inferring introgression from the observation of introgression-like variations in predominantly autogamous plants (where the probability of a hybrid reproducing by backcrossing is exceedingly low) is unwarranted. These effects would not be introgression *sensu* ANDERSON & HUBRICHT (1938) since no repeated backcrossing is involved. Although the taxa of subsection *Boreali-Americanae* spend a large portion of their seasonal life cycle at the cleistogamous phase, the chasmogamous phase is not sporadic, and the probability of a hybrid reproducing by backcrossing is high. Therefore, the hypothesis that the introgression might follow an initial hybridization event among some of these taxa is viable. The introgression-like effects described by Grant fit RIESEBERG & WENDEL's (1993) definition of introgression since they eliminated (following HEISER, 1973) the requirement for the specification of a specific crossing. GRANT (1981) also pointed out that in plant groups possessing a breeding system intermediate between outcrossing and inbreeding, hybrid reproduction can be expected to follow a mixture of pathways. This may be applicable to the situation among the taxa of subsection *Boreali-Americanae*.

4.5. Why Is Hybridization So Rampant Among the Taxa of *Viola* Subsection *Boreali-Americanae*?

Attempts at quantifying the extent of hybridization among the taxa of subsection *Boreali-Americanae* have not been made in the present study. Such attempts would require extensive and detailed studies of populations throughout the range of the taxa. Yet, frequent encounters in the field of populations consisting of putative hybrids, hybrid swarms, and *a posteriori* determinations of putative hybridity or introgression using SEM of the seed coat surface of seeds of plants that superficially resembled orthospecies support the observations of previous students of the subsection that hybridization among the taxa is rampant in the Eastern United States and Canada.

A number of previous students of the subsection and other authors have grappled with the question of why hybridization is so rampant among the taxa of subsection *Boreali-Americanae* in different contexts. BRAINERD (1906a) proposed a scenario to account for the lack of isolation between the taxa of subsection *Boreali-Americanae*. According to Brainerd, the northeastern United States was entirely covered with forests two or three centuries ago (this has not been proven to be correct, however). The clearing away of those forests by man has extended the range and habitats of *Viola*, which had been previously confined to habitats outside the primeval forests. As a result, the number of individuals has increased, and species which had been isolated before became cohabitant and thereby the opportunities to hybridize increased. RUSSELL (1954) outlined the following suggestions for the difficulties in delimiting species in *Viola* that are applicable to our question. First, the lack of genetic barriers to hybridization between closely related species throughout the genus. Second, the effects of human activities [drawing from ANDERSON's (1948) «hybridization of the habitat»]. Humans have greatly altered the natural habitats of plants over the greater part of the eastern United States, and consequently many species that were formerly separated were brought together. Edaphic situations that were formerly separated by large stretches of intermediate habitats have been brought into contiguity, and new habitats have been created. In addition, competition pressure has been released in habitats formerly closed, allowing hybrids to become established where before this was rarely possible. Third, the oscillating climates of the Pleistocene glaciations have instigated hybridization and introgression. VALENTINE (1962) applied CAMP's (1961) explanation of the distribution and breakdown of isolation between species of *Vaccinium* to the taxa of subsection *Boreali-Americanae*. He attributed the hybridization and the subsequent blurring of species boundaries to the repeated migrations of the species during the inter-glacial periods. Species were supposedly constricted in the South during the glacial maxima, and modified by hybridization and introgression, and then spread northwards to occupy new habitats.

The foregoing suggestions provided a number of important elements of a possible answer to our question. The Pleistocene glaciations have most likely affected the distributions of the taxa. They were followed by additional natural disturbances (e.g., floods, landslides, fire), human activities, or both. Human activities introduced disturbance in old habitats, and opened new ones. All three factors have, most likely, been facilitators in bringing into contact species that were formerly ecologically separated and other species that were also geographically separated. One of the best examples is the well documented (e.g., in BRAINERD, 1924) contact between *Viola pedatifida* that occupies prairies and *V. sororia* that occupies margins of open, mixed woods, shaded ledges, and disturbed sites. Many open prairies have become restricted to strips of land along borders of fields and railroads, and thereby have become physically closer to the habitats occupied by *V. sororia*. The decrease in distance between the habitats has probably increased the bi-directional movements of pollinators between the populations of the two species. It also allowed transfer of seeds of one species to the habitat of the other species by ants, and thereby increased the probability of outcrossing.

Furthermore, when we examine the general distributions of the taxa, we find that ecological and geographical barriers are still maintaining distinct and isolated populations of some of the taxa of the subsection. *Viola egglesonii*, endemic to cedar glades in the southeastern United States, is ecologically isolated from the rest of the taxa of the subsection. *Viola nuevo-leonensis*, growing in margins of chaparral and *Pinus-Quercus-Cercis* forests at relatively high elevations in northern México, is both ecologically and geographically isolated. There are no reports of hybridizations involving these species. Another example of geographic isolation is *V. nephrophylla*, which is the only species whose distribution extends to the western seaboard. There are numerous reports on hybridization of this species east of the Rocky Mountains where it has come into contact with other taxa. However, there are no reports on hybrids from the Pacific Northwest and northeastern California where populations of *V. nephrophylla* are geographically isolated. Similar geographic separation is found between the northeastern species *V. novae-angliae* and *V. septentrionalis*, and the species of the southeastern United States, *V. septemloba* and *V. villosa*. Even if we accept the arguments that the disruptions of geographic and ecological barriers promote hybridization, and that hybridization is likely in disturbed habitats, they are still not sufficient to explain why hybridization is so rampant among the taxa. Why do the taxa of subsection *Boreali-Americanae* hybridize more frequently than other taxa occupying the same habitats (including other taxa of *Viola*)?

The answer may be that the plants themselves are exceptionally pre-adapted for hybridization and introgression, and after hybridization or introgression have occurred, are also pre-adapted for maintaining and perpetuating some of the hybrids. These pre-adaptations are accomplished by the following factors. First, the plants have no genetic, structural or phenological barriers for outcrossing. Second, they have a wide spectrum of pollinators, and have not coevolved with specific pollinators. Consequently, gene flow can take place among all the taxa. Third, cleistogamy and vegetative reproduction ensure their perpetuation, and increase the number of fertile individuals (including hybrids and backcrosses) available for outcrossing and subsequent genetic recombination. Fourth, most hybrids are fertile, vigorous, and adaptable to habitat modifications.

4.6. The Consequences of Hybridization in *Viola* Subsection *Boreali-Americanae*

The literature on the evolutionary significance and consequences of hybridization and introgression is voluminous. The discussion that follows highlights some consequences that bear on the systematics of the taxa of subsection *Boreali-Americanae*.

A major consequence of hybridization among the taxa is stabilized hybrids. As outlined in the foregoing discussion, the taxa of subsection *Boreali-Americanae* are equipped with all three

modes of reproduction: chasmogamy, cleistogamy, and vegetative reproduction. Therefore, it should be no surprise that stabilized, homologous hybrids have evolved independently in many separate sites, persisted, and consequently mislead many taxonomists, who interpret them as orthospecies. RIESEBERG & WENDEL (1993) distinguished between recently hybridized or introgressant populations and stabilized introgressants that are reproductively isolated from their parental taxa. There is no evidence of reproductive isolation among the taxa and hybrids of the subsection.

What would be the destiny of the progeny of these hybrids? STEBBINS (1969) pointed out that the progeny of fertile hybrids will either degenerate or revert to their parents if they are unaided by natural selection, or if only selective forces that favor the parental genotypes are present. Furthermore, even if new habitats that change the action of natural selection are available, the emergence of derived populations with new adaptive properties will be greatly retarded by gene flow from their parents unless they achieve some degree of isolation. Most of the progeny of the fertile hybrids of subsection *Boreali-Americanae* do not degenerate (on the basis of Gershoy and Brainerd's studies, and observations made in this study). Some of them might revert to their parents if sufficient outcrossing with the parental plants is maintained, but the process might be slowed down compared to other plants due to their alternative modes of reproduction: cleistogamy and vegetative reproduction. Most of the reproductive portion of the life cycle of the plants is spent at the cleistogamous phase. Cleistogamy retains the sexual mechanism of reproduction – meiosis and recombination are taking place – and enables it to take place without dependency on pollinator availability. Even if outcrossing has not taken place during the brief chasmogamous phase in the spring, the perpetuation of these hybrids is maintained by the production of seeds by cleistogamy. Some of the hybrids in a population would hybridize with other hybrids, and produce hybrid swarms. To complicate the picture, some gene flow may occur between these hybrids and other taxa in adjacent populations depending on pollinator activity and the distances between populations.

Another possible consequence of hybridization is speciation through hybrid derivatives. A hypothesis on such possible evolution of *V. fimbriatula*, the only taxon in subsection *Boreali-Americanae* that does not display unique micromorphological characters on the seed coat surface, is presented in Chapter 6. Another hypothesis on taxa of this subsection was presented by STEBBINS (1950). He noted the various F₂ segregates bearing blades with different degrees of dissection that were illustrated by BRAINERD (1924) (e.g., segregates of the F₁ hybrid of *Viola pedatifida* and *V. sagittata*). He suggested that by comparing these segregates with the stable and constant species in the eastern United States bearing "unusual leaf forms," such as *V. palmata*, *V. brittoniana*, *V. triloba*, *V. stoneana*, and *V. viarum*, one can hypothesize that these species have been derived by stabilization of hybrid derivatives. Therefore, there is a substantial reason for suggesting that the number of stable, recognizable species in subsection *Boreali-Americanae* has been considerably increased by hybridization. The micromorphological data (GIL-AD, 1995) and the correlation of this data with macromorphological and other data (see Chapter 6) revealed that three of the species that Stebbins listed, *V. palmata*, *V. stoneana*, and *V. viarum*, are of putative hybrid origin, and therefore his prediction was correct, in part. Morphological data do not provide evidence to support the hypothesis that the other two species (representing the taxa possessing divided or lobed blades) have been derived from stabilized hybrids. Divided and/or lobed blades have evolved also in the subsections *Chrysanthae* and *Nudicaules* of *Viola* section *Chamaemelianum*, and in subsection *Pedatae* of section *Plagiostigma*. It is more likely that the taxa of subsection *Boreali-Americanae* that possess divided or lobed blades are orthospecies that have evolved by divergence, and not as derivatives of stabilized hybrids. Nevertheless, the final evaluation of Stebbins' hypothesis, as well as the hypothesis concerning the origin of *V. fimbriatula*, await testing by molecular techniques.

4.7. The Nomenclatural Treatment of Hybrids and Introgessants

The frequent occurrence of hybrids and introgressants of the taxa of the subsection raise the question of how they should be treated and subsequently, how their specimens should be annotated.

The nomenclature of hybrids is covered in Appendix I of the International Code of Botanical Nomenclature (ICBN) (GREUTER & *al.*, 1994). Article H.4 (page 85) states that “When all the parent taxa can be postulated or are known, a nothotaxon is circumscribed so as to include all individuals (as far as they can be recognized) derived from the crossing of representatives of the stated parent taxa (i.e., not only the F₁ but subsequent filial generations and also backcrosses and combinations of these). There can thus be only one correct name corresponding to a particular hybrid formula; this is the earliest legitimate name...in the appropriate rank..., and other names to which the same hybrid formula applies are synonyms of it.” Thus, when sufficient evidence (e.g., micromorphological or molecular data) is available to support the postulation of the parents of a given hybrid belonging to subsection *Boreali-Americanae*, the published names (see under the discussion of the appropriate orthospecies in Chapter 6, and also Appendix B) for the particular hybrid combination should be used. If a name has not been published, a hybrid formula should be designated (WAGNER, 1983; GREUTER & *al.*, 1994). When hybridity can be postulated (e.g., when the plant’s suite of characters does not match that of any orthospecies), but sufficient supporting evidence is not available to allow positive identification of the putative parents, it is better to designate the plant as a putative hybrid, and leave it unnamed. Such procedure would encourage subsequent researchers to seek additional evidence. In addition, it would discourage a common curatorial practice in many herbaria of filing specimens of such plants with one of the stated parents regardless of their morphology. Frequently, this is followed by annotation of such specimens as orthospecies, and consequently, great disarray.

The problem of designating a backcross that closely resembles one of its parents or an introgressant is of special concern when dealing with hybrids of subsection *Boreali-Americanae*. WAGNER (1983) suggested that a hybrid would be classified with its parent if it is so close and cannot be separated from it. This is a workable solution when the putative parents are widely separated, which should be practiced in other taxa. However, in subsection *Boreali-Americanae*, where species are closely related, interfertile, and separated by a relatively small number of characters, and where macromorphological characters (in most taxa) are not sufficient to be used alone to delimit taxa, such a practice would reinstate the obliteration of the species boundaries. When evidence for introgression is available to indicate that, the plant should be annotated properly.

HARDIN (1958) reviewed the many different methods of annotation of introgressants. He pointed out that the use of the symbols > and < as more or less arrows indicating the direction of introgression, or gene flow, might add to confusion, since these symbols are more associated with their mathematical usage. He recommended that the dominant component (the recurrent parental species *sensu* ANDERSON, 1949) would appear first after the genus name or its abbreviation, and would then be followed by the > symbol and the epithet of the second component. For example, if *Viola affinis* introgressed into *Viola cucullata*, the product of the introgressive hybridization would be annotated as *Viola cucullata* > *affinis*. If the epithets are kept in alphabetical order, e.g., *Viola affinis* < *cucullata*, the formula might be confusing, and the specimen might be filed under the first epithet rather than under the epithet of the dominant component in this example. TERRELL (1963) favored the usage of the greater than and smaller than symbols, as advocated by Hardin, but suggested that these symbols would be used with a dash to indicate the direction of intergradation (e.g., *Viola cucullata* >–*V. affinis*). Hardin’s and Terrell’s suggestions have not been adopted and incorporated into the ICBN (WAGNER, 1983).

An alternative method would be to use the symbol of a simple arrow pointing backwards: ← to designate the direction of introgression. This symbol is widely available in most of the contemporary word processing computer-programs, and would not be confused with mathema-

tical symbols. The dominant component would follow the name or abbreviation of the genus, and would be followed by the arrow, and then by the epithet of the minor component. Using the same example, this notation would be written as *Viola cucullata* ← *affinis*, and would be read: *Viola cucullata* introgressed by *Viola affinis*. Thus, the notation of the documented case of putative introgression presented in GIL-AD (1995), is *Viola nephrophylla* ← *novae-angliae*, and it reads: *Viola novae-angliae* introgressed into *Viola nephrophylla*. When there is some evidence for putative introgression, but it is inconclusive (e.g., when foreign micromorphological structures are detected on the seed coat surface, but they can be attributed to more than one orthospecies) a question mark can be interpolated between the arrow and the binomial of the putative minor component to indicate uncertainty.