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# AN ANALYSIS OF INTROGRESSION IN A POPULATION OF STEMLESS WHITE VIOLETS*

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Speciation in the stemless white violets has generally been recognized as complicated. There are numerous taxa; variability from plant to plant in any one population is frequently high; and differences from one population to another are sometimes of uncertain interpretation. Careful field and herbarium work (Fernald, 1950, and Russell, 1953) has cleared up some of the complexities but considered in the large has raised as many problems as it has solved.

On two occasions I have had the opportunity to make brief but intensive studies of local populations, once in Minnesota with Dr. Norman Russell, once in Pelham, Massachusetts, with Dr. R. P. Levine and the biology majors from Amherst College. On the latter trip, two days were spent examining variable local populations of *Viola pallens* in the Pelham Hills. They were studied in the field; a critical mass collection was made; the variation patterns were analyzed in the laboratory, first morphologically, and then by simple statistical and semigraphical devices. From the data, introgression with a second taxon was established and a detailed technical description of it was drawn up by the method of extrapolated correlates. A second field trip confirmed the presence of just such a violet in the same area (the Pelham Hills), but since it was just barely coming into flower further studies of it were not possible. Neither species grows in the neighborhood of St. Louis, nor could readily be grown there because of climatic and soil differences. No further work with the problem is planned, but the data and the analyses are being put on record since they illustrate certain phenomena of introgressive variability which are of general importance. I am indebted to Dr. Levine for the opportunity of making this analysis and to a score of his colleagues and students for technical assistance.

After the mass collection was brought back to the laboratory, flowers from each plant were placed in numbered culture dishes to prevent withering, and special features of the variation pattern were assigned to groups of two to four students, first for morphological analysis and then for measurement. As soon as the measure-

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ments were completed, frequency distributions of each variable and scatter diagrams indicating the relationships between variables were prepared. One pair of students assisted in coordinating the work of the entire laboratory, seeing that each group studied each plant and that the measurements and grades were added to the master list. In this way the equivalent of several days of technical work was completed in one afternoon.

Vegetative propagation is well developed in *Viola pallens*. Nearly all the plants examined bore several flowers and a few of them made large mats with numerous blooms. This was taken advantage of in two ways: (1) Examination of the variation within and between plants made it possible to choose characters which were relatively independent of environmental effects. (2) In selecting flowers for measurement, great care was taken to choose a specimen which was typical of the plant on which it was borne. This minimized the effects of insect attack and other injuries.

It was soon apparent that the most conspicuous plant-to-plant variable in the population of *Viola pallens* was the amount of color on the lower petals. Repeated experience with such problems in various genera of plants and animals has demonstrated that by persistence and biological acumen a vague difference of this sort can nearly always be broken down into numerous more-primary variables each of which can then be dealt with quite precisely. Various means were tried out for recording and measuring the plant-to-plant differences in colored veins in terms of such primary features as number and position of veins, branching of veins, width of veins, deposition of color between the veins, and the like. In the time available it was possible to resolve the variation in color into the primary variables shown in fig. 1.

Other obvious variables in the population were leaf shape, leaf color, leaf pubescence, and the clubbed hairs towards the base of certain petals. It was not possible to find an effective way of measuring the latter variable. The hairs were varying in size, in the closeness of their spacing, and in the pattern of their distribution on the petals. In some flowers they tended to be in regular lines, in others not. Sometimes they were more dense in certain areas, sometimes not. There were obviously several different variables at work here, but in the time available it was not possible to pin them down. One of the basic difficulties was that *Viola pallens* is typically without any such hairs on the wing petals, and there is therefore no direct method by which we can determine its basic hair pattern (a very different thing from hair presence). This illustrates a major point to be kept in mind in the analysis of introgressive variability. Glabrous species, when hybridizing with pubescent species, nearly always bring in strong hair patterns of their own which of course are invisible in the species in which they originated. Similarly, white-flowered species when hybridizing with species whose flowers are colored nearly always bring in color modifiers and color patterns which were invisible or virtually so in the species whence they came. White-flowered species, for instance, are frequently genetically blue, though they may show no color or only a few lines or flushes of dark blue. If such a species is crossed to one with bright magenta-pink

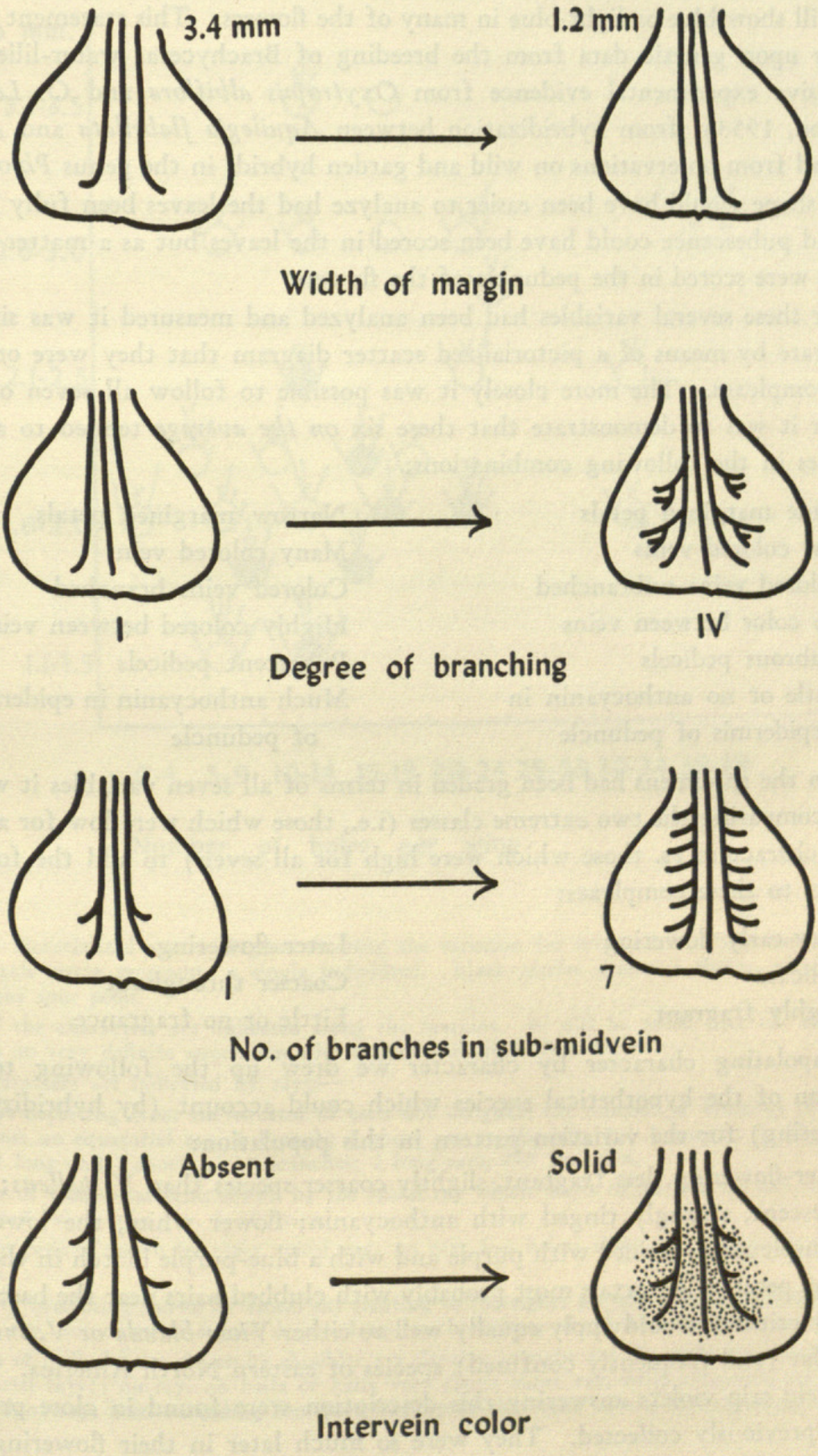


Fig. 1. The four variables which were responsible for the variation in color in the spur petals. The extremes within the population are shown somewhat diagrammatically. The spur petals are shown as if they were always the same size and shape; actually they varied a great deal for these characters.

flowers, and this hybrid back-crossed to the white, the resulting three-quarter bloods will show blue or light blue in many of the flowers. This statement is based primarily upon genetic data from the breeding of Brachyceras water-lilies, from introgressive experimental evidence from *Oxytropus albiflora* and *O. Lambertii* (Anderson, 1953), from hybridization between *Aquilegia flabellata* and *A canadensis,* and from observations on wild and garden hybrids in the genus *Phlox.*

Leaf shape would have been easier to analyze had the leaves been fully mature. Color and pubescence could have been scored in the leaves but as a matter of convenience were scored in the peduncle of the flowers.

After these several variables had been analyzed and measured it was simple to demonstrate by means of a pictorialized scatter diagram that they were organized in two complexes. The more closely it was possible to follow all seven of them, the easier it was to demonstrate that these six *on the average* tended to associate themselves in the following combinations:

| | |
|---|---|
| Wide margined petals | Narrow margined petals |
| Few colored veins | Many colored veins |
| Colored veins unbranched | Colored veins branched |
| No color between veins | Highly colored between veins |
| Glabrous pedicels | Pubescent pedicels |
| Little or no anthocyanin in epidermis of peduncle | Much anthocyanin in epidermis of peduncle |

When the specimens had been graded in terms of all seven variables it was possible by comparing the two extreme classes (i.e., those which were low for all seven of these characters vs. those which were high for all seven) to add the following characters to these complexes:

| | |
|---|---|
| Very early flowering | Later flowering |
| Delicate | Coarser throughout |
| Highly fragrant | Little or no fragrance |

Extrapolating character by character we drew up the following technical description of the hypothetical species which could account (by hybridizing and back-crossing) for the variation pattern in this population:

A later-flowering, less fragrant, slightly coarser species than *V. pallens;* peduncles pubescent, strongly tinged with anthocyanin; flower white, the lower three petals conspicuously veined with purple and with a blue-purple blotch in the center of the keel petal; wing petals most probably with clubbed hairs near the base. Much of this description would apply equally well to either *Viola blanda* or *V. incognita,* two similar (and frequently confused) species of eastern North America. On the second field trip violets answering this description were found in close proximity to those previously collected. They were so much later in their flowering season that the details of their color pattern could not yet be precisely determined.

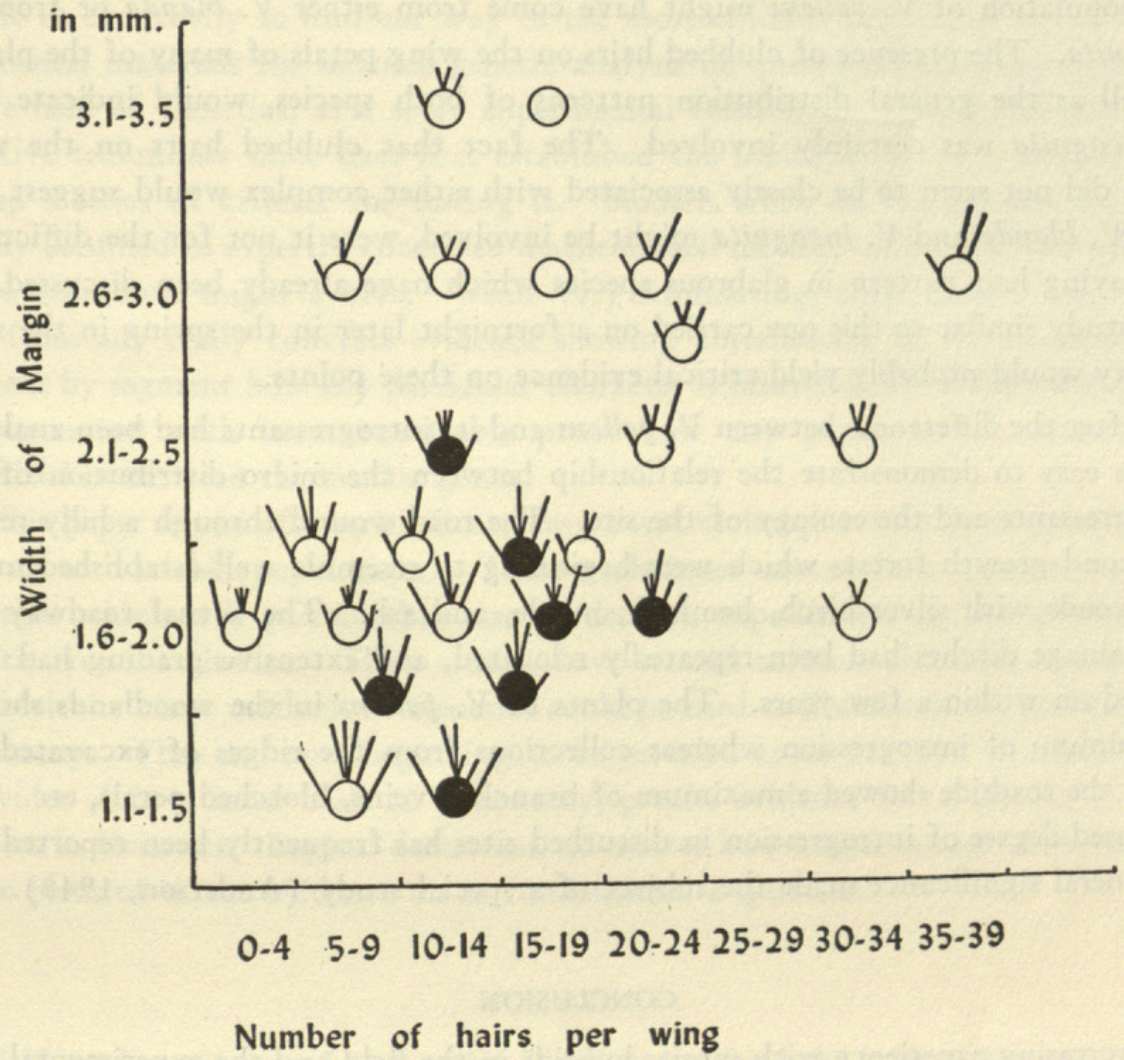


Fig. 2. Pictorialized scatter diagram showing the variation for seven characters in the population studies. Each circle represents a single individual. Black circles denote a flower with a heavy blotch on the spur petal.

Two of the characters are indicated along the margins. It will be noted that the number of hairs shows no very definite association with any of the other characters.

Five characters are indicated by rays:—

The rays departing from the equator of each dot designate the number of branches in the submedian veins: no equatorial ray, 1 branch; 1 short ray to the left, 2–3 branches; 2 short rays, 4 branches; 1 long and 1 short ray, 5 branches; 2 long rays, 6–7 branches.

Number of wing-petal veins shown by the apical ray which slants to the left: no ray, 0–3 veins; short ray, 4–7 veins; long ray, 8–10 veins.

Hairs on pedicel shown by erect apical ray: no ray, 1–4 hairs; short ray, 5–9 hairs; long ray, 10–13 hairs.

Degree of branching shown by apical ray slanting to the right: no ray, branching of first degree only; short ray, branching of second degree; long ray, branching of third or fourth degree.

Position of pedicel hairs shown by shoulder ray slanting to right (at the right between the apical and equatorial rays): no ray, no hairs or hairs very faint; short ray, hairs extending up to crook of pedicel; long ray, hairs extending fom part way up to the crook of the entire pedicel.

From the data presented here, the introgression responsible for the variation in this population of *V. pallens* might have come from either *V. blanda* or from *V. incognita.* The presence of clubbed hairs on the wing petals of many of the plants, as well as the general distribution patterns of both species, would indicate that *V. incognita* was certainly involved. The fact that clubbed hairs on the wing petals did not seem to be closely associated with either complex would suggest that both *V. blanda* and *V. incognita* might be involved, were it not for the difficulties in assaying hair pattern in glabrous species which have already been discussed. A field study similar to this one carried on a fortnight later in the spring in the same locality would probably yield critical evidence on these points.

After the differences between *V. pallens* and its introgressants had been analyzed it was easy to demonstrate the relationship between the micro-distribution of the introgressants and the ecology of the site. The road wound through a hilly region of second-growth forests which were beginning to resemble well-established mixed hardwoods with silver birch, hemlock, maple, and ash. The actual roadway and its drainage ditches had been repeatedly relocated, and extensive grading had been carried on within a few years. The plants of *V. pallens* in the woodlands showed a minimum of introgression whereas collections from the ridges of excavated soil along the roadside showed a maximum of branched veins, blotched petals, etc. This increased degree of introgression in disturbed sites has frequently been reported and its general significance made the subject of a special study (Anderson, 1948).

## CONCLUSION

Increasing experience with species hybrids in the field and the experimental plot has shown that what looks like a simple difference between two species can often be broken down into a number of more primary ones, each of which is itself apparently multifactorial. In this example the difference between a heavily marked spur petal and a lightly marked one can be demonstrated as resulting from the following more basic differences:

Number of colored veins
Branching of veins
Restriction or non-restriction of colored veins to center of petal.
Interveinal blotching.

In *Adenostoma* (Anderson, 1952, 1954) dense versus open panicles can be demonstrated as resulting from the following more basic differences, each of which behaves as if it were multifactorial and is only loosely associated with any one of the others:

Long vs. short internodes
Non-telescoped vs. telescoped internodes
No evident tertiary branches vs. many evident tertiary branches
One flower per node vs. several flowers per node.

The field analysis of such examples of introgression as that just described should enable us eventually to find our way to the most efficient techniques and best experimental materials for detailed genetic analysis of quantitative characters. This entire field of Genetics, as a truly experimental science, has scarcely advanced in effective techniques since East first established the multiple-factor hypothesis and set up a series of criteria for testing it. Modern work on the subject has been largely confined to expertly contrived mathematical models, indicating how quantitative characters might evolve. While very stimulating, these models are a long way from any really concrete evidence showing chromosome by chromosome and segment by segment how any particular character is inherited or, on the other hand, demonstrating with incontrovertable proof how any particular chromosome is organized in between the marker genes.

This paper is one of a series illustrating exact techniques for the morphological analysis of variable populations. These techniques are at last approaching the precision which will permit their use in decisive experiments on the genetics of quantitative inheritance. These studies have seemed to most observers as a means by which the exact methods of Genetics could be used to illuminate the problems of Taxonomy. They are now beginning to be revealed as an attempt to refine from the wider observational basis of Taxonomy, precise evidence for analyzing a basic problem in Genetics. Logical deduction, no matter how acute, cannot serve forever in the place of direct evidence on so fundamental a question.

## SUMMARY

Field studies of a population of *Viola pallens* resolved the bulk of the variability into two complexes, one of which is *V. pallens* and the other a later, slightly coarser, and more deeply pigmented species. The purple petal spot of this latter complex is shown to result from the following more primary variables, each of which is apparently multi-genic:

| | |
|---|---|
| Wide marginal area without veins | Narrow marginal area |
| Few colored veins | Many colored veins |
| Few branched veins | Many branched veins |
| No interveinal color | Heavy interveinal color |

The bearing of such studies on the genetics of quantitative inheritance is specifically pointed out.

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