

CYTOGENETIC STUDIES IN PAEONIA I.
THE COMPATIBILITY OF THE SPECIES AND THE
APPEARANCE OF THE HYBRIDS

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INTRODUCTION

SINCE 1916, the senior author has carried on an extensive series of crossing experiments in the genus *Paeonia*, with the object both of obtaining new horticultural forms and of securing evidence concerning the interrelationships of the species and the processes of evolution within the genus. Some accounts of the hybrids have already been published (SAUNDERS 1928, 1933a, 1933b). The junior author began cytological work on the species and hybrids in 1932, continuing that started by the late Dr. G. C. HICKS, whose work along with additional data obtained by the junior author has also been published (HICKS and STEBBINS 1934). Since that time the cytological as well as the morphological study of the hybrids has progressed steadily, except that it was somewhat interrupted by the departure of the junior author for California in 1935.

The following limitations prevent a cytogenetic study of this genus comparable to that carried on in other genera, such as *Crepis*, *Nicotiana*, and *Datura*. Peony seeds are slow of germination, and the plant takes several years to mature; from the date of hybridization to the season of first blooms is usually a gap of six or seven years. This makes it difficult for one individual to raise many successive generations. An added difficulty is that most interspecific hybrids in *Paeonia* are completely sterile for at least two or three years after they first begin to bloom; older plants of almost all the hybrids, when they have established themselves as strong clumps, set occasional seeds, usually not more than one or two to an entire plant. The size of the plants and the cost of their care through so long a period of years also make large scale experimentation for statistical data impracticable. There are in this collection at present several thousand hybrid plants of blooming age, representing more than thirty distinct strains. The range of attempted crossings was much wider, but as was to be expected many of them persistently failed to produce seed.

SOURCE OF THE MATERIAL

In any genus that contains a number of cultivated species, interspecific relationships are likely to be obscured by selection and hybridization under cultivation. For this reason, particular attention has been given to the source of the material used, and every effort has been made to determine

the genetic similarity of the various clones used to wild biotypes. Fortunately for this purpose, the clones of *Paeonia* are very long lived, and the species are relatively infrequently propagated by seed, so that forms which have been in cultivation for many years are only a few generations removed from their wild prototypes. Furthermore, with a few notable exceptions, interspecific hybrids in the genus are so sterile that they can readily be identified, and in most cases would be very unlikely to contaminate the pure species by backcrossing. Hence the cultivated clones that differ from the wild stocks of the same species are in most cases either hybrids between different varieties or biotypes, or strains resulting from the repeated selection of favorable mutations. The most notable exceptions to this rule, the tetraploid European species and garden forms, have for this reason been given relatively little attention in the general study of interspecific relationships in the genus, although significant evidence has been obtained as to the probable mode of origin of the group as a whole. This will be presented more fully in a later paper.

The following table describes the origin and character of the clones most frequently used for hybridization. The letter **r** after the designation of the source indicates that roots were obtained, the letter **s** that seed was received, from which seedlings were grown by the senior author. The approximate date of introduction into cultivation of each form is given, based on such published records as are available, while the number of generations which the particular clone used in hybridization is removed from the wild plant is estimated from notes received from the various horticultural firms, and from the frequency with which the form is grown and propagated. In the final column the abbreviation "herb." indicates that the clone used has been matched with authentic herbarium specimens of wild plants, while "ill." indicates that it compares closely with a published illustration accompanying the original or another authentic description of the form. The taxonomic status of the forms designated with a star (*) is discussed elsewhere (STEBBINS 1938). The name Van Tubergen refers to C. G. VanTubergen Ltd., Haarlem, Holland; Vilmorin, to Vilmorin et Cie., Paris; Glasnevin to Glasnevin Botanical Gardens, Dublin Ireland; and Barr to Barr and Sons, Ltd., London.

COMPATIBILITIES OF THE SPECIES

Although the number of matings made of any particular cross was in most cases not large enough so that accurate quantitative data could be secured concerning the degree of success, it was possible to group them into four classes:

- I. Hybrids obtained as in crosses between individuals of the same variety (about 80–100 percent seed setting).

NAME	SOURCE	NATIVE HABITAT	DATE INTRODUCED	GENERATIONS FROM WILD PLANT	COMPARED
<i>P. Delavayi</i> Franch. var. <i>lutea</i> (Franch) Finet & Gagnep.	Van Tubergen—r	S.W. China	1885	2-3	herb.
<i>P. suffruticosa</i> Andr.	various	N.W. China	?	∞	o
<i>P. albiflora</i> Pall.					
The Bride (single)	various	N. China	?	∞	
Clairette (single)	various	Manchuria	?	∞	o
Whitley major (single)	various	Siberia	?	∞	o
Primivere (double)	various		?	∞	o
James Kelway (double)	various		?	∞	o
<i>P. anomala</i> L.	Highland Park	Siberia	1750	?	herb. ill.
typical	Rochester, N.Y.—r				
<i>Veitchii</i> *	Vilmorin—r	W. China	1909	1-2	herb.
<i>Woodwardii</i> *	Vilmorin—r	W. China	1920	1-2	herb.
<i>Beresowskii</i> *	Glasnevin—r	W. China	1920	1-2	herb.
vars.	Leningrad B.G.—s H.A. Hesse Weener, Germany	W. China	?	?	herb.
<i>P. Emodi</i> -Wall.	Glasnevin—r	Himalaya	1840-		
	Hyde Park		1860	2-6	herb. ill.
	London—s				
<i>P. tenuifolia</i> L.	private garden—r	S.E. Europe	1750	?	herb. ill.
<i>P. triternata</i> Pall.					
typical	Barr—r	S.E. Europe	1810	?	herb. ill.
<i>Mlokosewitschii</i> (Lomak)*	Van Tubergen—r and own—s	Caucasus	1895	2-3	ill.
<i>P. Broteri</i> Boiss*	Barr—r	Spain, Portugal	1880- 1885	2-5	herb. ill.
<i>P. obovata</i> Maxim	Sakata & Co. Yokohama—s	Japan	1890- 1900	1-2	herb.
<i>P. tomentosa</i> Stapf. (= <i>P. macrophylla</i> of auth. not Lomak., cf. Stapf 1931)	Van Tubergen—r	Caucasus	1900- 1908	3-5	ill.
<i>P. coriacea</i> Boiss	Cent. Expt. Farm, Ottawa,—r Canada	S. Spain, Morocco	1870-1890	?	o
<i>P. Willmanniana</i> Hartw.					
typical	Van Tubergen	N. Persia	1840-1845	3-4	o
<i>Willmottiae</i> (Stapf)*	J. C. Allgrave Langley, England—r	W. China	1908	1	o
<i>P. corallina</i> Retz.*	Vilmorin—r	S. Europe	?	∞	herb. ill.
<i>P. officinalis</i> L.					
single crimson	own—s	Europe	?	∞	o
<i>rubra plena</i>	various—r	Europe	?	∞	o
Otto Froebel	various—r	Europe	?	∞	o

II. Hybrids obtained with some difficulty (2-80 percent seed setting).

III. Hybrids obtained with great difficulty (less than 2 percent of normal seed setting).

IV. Hybrids not obtained.

Compatibilities between the diploid species. These are illustrated by the chart, figure 1. This shows that the degree of compatibility between two forms is a good criterion for the delimitation of species and subgenera. In every case where the compatibility was perfect, the parents were closely related and the hybrid fertile, indicating that the cross was between different varieties of the same species. On the other hand, crosses between

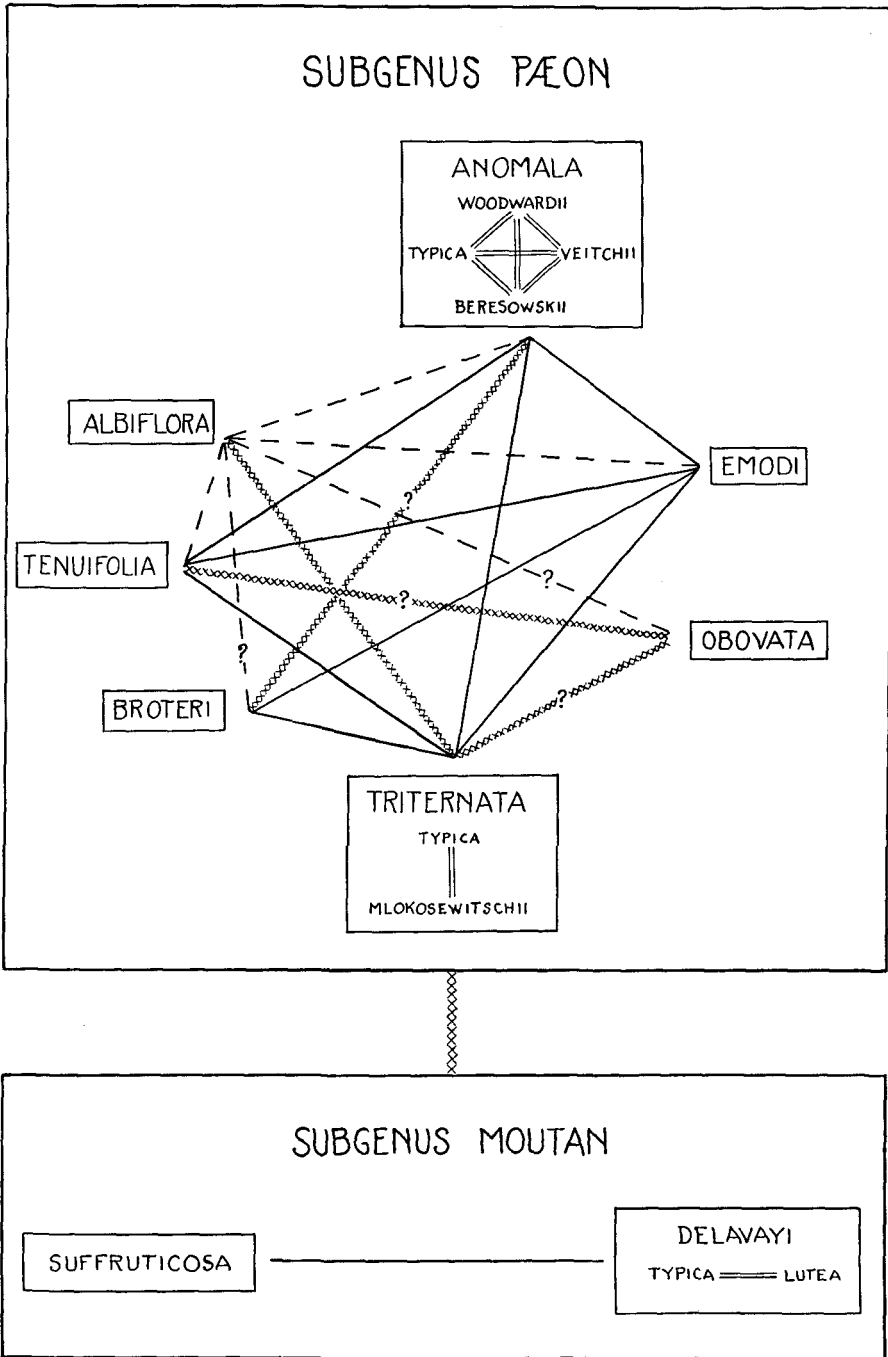


FIGURE 1. (See opposite page for description.)

members of different subgenera have never been successful, while in only one case (*P. albiflora* × *P. triternata Mlokoewitschii*) has a cross between two diploid members of the same subgenus been unsuccessful after a large number of attempts.

On the other hand, compatibility has been of no value whatever in determining the interrelationships within a single subgenus. For instance, *P. anomala* is fairly similar in leaf, sepal, and other floral characteristics to *P. albiflora*, but crossing between the two is very difficult; on the other hand it is relatively easy between *P. anomala* and *P. triternata Mlokoewitschii* or *P. tenuifolia*, both of which are much more different in their appearance from *P. anomala* than is *P. albiflora*.

Compatibilities between the tetraploid species. The tetraploid species, in contrast to the diploid, are uniformly perfectly compatible with each other, and most of them intercross naturally when placed side by side in the garden. Although many of the hybrids thus obtained are partially or wholly sterile, this ability for free intercrossing is undoubtedly an important cause of the taxonomic complexity of these species.

Compatibilities between diploid and tetraploid species. Only two tetraploid species groups have been tested sufficiently, *P. officinalis* (including the various varieties and horticultural forms mentioned above) and *P. tomentosa*. The compatibility of these two with the various diploid species is given in table 1. A striking fact brought out by this table is that *P. albiflora*, which is very difficult to cross with the other diploid species, crosses relatively easily with the tetraploids. In this series also compatibility was not the greatest between species closely similar morphologically. For instance, *P. triternata Mlokoewitschii* and *P. tomentosa*, two species taxonomically very close to each other, cross with such difficulty that out of 54 pollinations only eight F₁ plants have been obtained and yet *P.*

FIGURE 1.—Chart illustrating the compatibilities of the diploid species of *Paeonia*. With the exception of *P. Emodi* and *P. obovata*, each species within the rectangle representing the subgenus *Paeon* occupies a position between its two closest relatives; hence the closest taxonomic relationship is between adjacent species, and the greatest divergence is between species opposite each other. The lines between the rectangles representing subgenera, species, and subspecies are explained as follows:

===== Hybrids obtained as easily as in crosses between individuals of the same subspecies, fertile.

----- Hybrids obtained with some difficulty (2 percent or more of normal seed setting) and sterile.

- - - - Hybrids obtained with great difficulty (less than 2 percent of normal seed setting), sterile.

XXXX Hybrids not obtained.

A ? indicates that the number of attempts as yet made is insufficient to determine definitely the nature of the cross. The absence of a connection between two rectangles indicates that the cross between these species has not been attempted.

tomentosa crosses relatively easily with the very distantly related species, *P. albiflora*. Furthermore, the compatibility of *P. albiflora* with some races of *P. officinalis*, such as the horticultural var. *lobata*, is very high, while other races of *P. officinalis* can be crossed only with great difficulty. This difference is illustrated in table 1, where *P. albiflora* appears in several places under *P. officinalis*.

The other unexpected fact is the behavior of reciprocal crosses between diploid and tetraploid species of this genus. Practically all such crosses

TABLE 1

Table illustrating the compatibilities of two tetraploid species, *Paeonia officinalis* and *P. tomentosa*, with the diploid species.

	<i>officinalis</i>	<i>tomentosa</i>
I. Hybrids easily obtained	<i>albiflora</i> *	
II. Hybrids obtained with some difficulty	<i>albiflora</i> *	<i>albiflora</i> ♀
III. Hybrids obtained with great difficulty	<i>albiflora</i> * <i>tenuifolia</i> <i>triternata</i>	<i>triternata</i> <i>albiflora</i> ♂
IV. Hybrids not obtained	<i>Delavayi</i> <i>suffruticosa</i> <i>anomala</i>	<i>anomala</i> <i>tenuifolia</i> <i>Delavayi</i> <i>suffruticosa</i>
Not attempted	<i>Emodi</i> <i>obovata</i> <i>Broteri</i> <i>Brownii</i>	<i>Emodi</i> <i>obovata</i> <i>Broteri</i> <i>Brownii</i>

* See text for explanation.

have been made with a tetraploid species as the pollen parent, and a diploid, usually *P. albiflora*, as the ovulate parent. The results of reciprocal hybridization show that the cross between *P. albiflora* and *P. tomentosa* can be made much more easily with the former as the ovulate parent (SAUNDERS 1933b). This is contrary to the rule in most other genera (WATKINS 1932, and many other cases). In the other diploid-tetraploid cross listed, *P. albiflora* × *officinalis*, there was no significant reciprocal difference in compatibility. No explanation for this difference in behavior between the *P. tomentosa* and *P. officinalis*, or for the unusual situation in the hybrid between the former and *P. albiflora* can as yet be given. It may be noted, however, that the seeds of *Paeonia* developed a copious endosperm, so that the disharmony between embryo and endosperm development postulated by WATKINS would be expected to hold here.

APPEARANCE OF THE HYBRIDS

In the appearance of the hybrid populations in general, the variability of the F₁ plants from the same cross is most striking. This is particularly true in crosses which involve as one parent *P. albiflora*, *P. suffruticosa*, or some member of the complex of *P. officinalis*. Since these three species have all been cultivated for centuries and possess a large number of horticultural varieties, the variation in the F₁ populations probably reflects the heterozygous character of one or both parents. Nevertheless, sometimes

TABLE 2

The character of the sepals in diploid and tetraploid species and their hybrids. The character of the innermost sepal is expressed by the presence (+) or the absence (-) of a strong midrib which persists to its apex, and the length of the terminal mucro, if present (drawings, in figures 2 and 3).

SPECIES OR HYBRID	SOMATIC CHROMOSOME NUMBER	USUAL NUMBER OF SEPALS	INNERMOST SEPAL	
			CHARACTER OF MIDRIB	LENGTH OF MUCRO
<i>P. tenuifolia</i>	10	7	-	0
<i>P. triternata Mlokoewitschii</i>	10	4	-	0
<i>P. anomala Veitchii</i>	10	6	+	5-7 mm
<i>P. anomala Woodwardii</i>	10	6	+	1.5-2 mm
<i>P. albiflora</i>	10	7	+	0.5-1.5 mm
<i>P. officinalis</i>	20	5	+	0
<i>P. tomentosa</i>	20	4	-	0
<i>P. tenuifolia</i> × <i>an. Veitchii</i>	10	7	+	2 mm
<i>P. tenuifolia</i> × <i>an. Woodwardii</i>	10	7	+	1.5-2 mm
<i>P. tenuifolia</i> × <i>albiflora</i>	10	7	+	1-1.2 mm
<i>P. tri. Mlokoewitschii</i> × <i>P. an. Veitchii</i>	10	6	+	1.5 mm
<i>P. tri. Mlokoewitschii</i> × <i>P. an. Woodwardii</i>	10	6	+	0.2 mm
<i>P. albiflora</i> × <i>officinalis</i>	15	6-7	18+ 16-	0-0.7 mm
<i>P. albiflora</i> × <i>tomentosa</i>	15	4-5	-	0

the variation within a single population transcends that of either of the parent species. For instance, among the F₁ hybrids of *P. albiflora* × *tomentosa*, occasional dwarf plants with strap-shaped petals appear. These may be sibs of perfectly normal plants, or even of plants which show "hybrid vigor" in the great size of their leaves. A similar situation prevails in the crosses of *P. albiflora* × *P. officinalis*, *P. albiflora* × *P. tenuifolia*, and *P. Delavayi* × *P. suffruticosa*. In crosses between species possessing few horticultural varieties, and presumably more homozygous, the F₁ populations are much more uniform.

Although the F₁ hybrids of each cross are intermediate between their parents in nearly every characteristic, definite trends of dominance and recessiveness could be noted in a few, as follows:

The sepal characters. As is explained in another publication (STEBBINS 1938), the difference between the sepals of the species is one of the most significant taxonomic characters in the genus, and shows a progression from a phylogenetically primitive to an advanced condition. Since this character may be most accurately represented by the number of sepals and the character of the innermost sepal, a table showing these in the crosses involving sepal differences is presented in table 2.

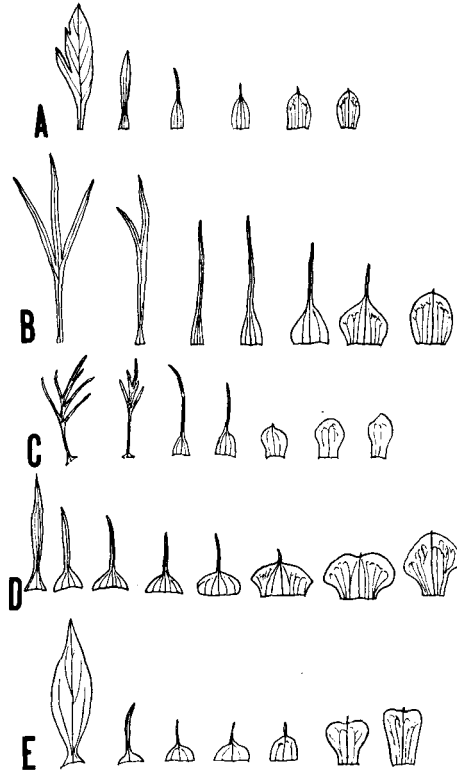


FIGURE 2.—The sepals of: A. *Paeonia anomala* Woodwardii, B. *P. tenuifolia* × Woodwardii, C. *P. tenuifolia*, D. *P. albiflora* × *tenuifolia*, E. *P. albiflora*. All $\times \frac{1}{2}$.

This table shows that, while the sepals of the hybrids are to a certain extent intermediate between those of their parents, all of the diploid hybrids are more nearly like their more primitive parent, that is, that one which retains the appendage on the innermost sepal. Hence we may conclude that the genes for the more primitive type of sepals are more or less, though not completely, dominant over those for the more advanced type. The various sepal types involved are illustrated in figure 2 and elsewhere (STEBBINS 1938).

Noteworthy exceptions to this rule are found, to be sure, in crosses involving one of the tetraploid species, for example, *P. officinalis* or *P.*

tomentosa. In the *P. albiflora* × *tomentosa* series, although about fifty F₁ plants were examined, none was found in which the midrib of the innermost sepal extended to its apex (fig. 3). One may conclude from this fact that the two diploid genomes which make up the tetraploid set of *P. tomentosa* both contain genes for the absence of a midrib and mucro, and that therefore the double number of genes for this character present in the hybrid has overcome the normal dominance of the midrib-mucro genes. The hybrids between *P. albiflora* and *P. Wittmanniana* as well as *P. coriacea*, two other tetraploid species closely related to the diploid types which lack the midrib and mucro, are quite similar, although in these cases only two or three plants were examined.

TABLE 3
The inheritance of side buds.

PARENTS		HYBRID	
Pollen	Ovulate		
<i>P. lutea</i>	+ × <i>P. suffruticosa</i>	—	(+)
<i>P. tenuifolia</i>	— × <i>P. anomala Veitchii</i>	(+)	—
<i>P. tenuifolia</i>	— × <i>P. anomala Woodwardii</i>	+	(+)
<i>an. Veitchii</i>	(+) × <i>P. triternata Mlokoewitschii</i>	—	—
<i>an. Mlokoewitschii</i>	— × <i>an. Woodwardii</i>	+	+
<i>P. albiflora</i>	+ × <i>P. tenuifolia</i>	—	+
<i>P. albiflora</i>	+ × <i>P. officinalis</i>	—	+
<i>P. albiflora</i>	+ × <i>P. coriacea</i>	—	—
<i>P. albiflora</i>	+ × <i>P. tomentosa</i>	—F ₁	+, —
		F ₂	+, —

The F₁ plants of *P. albiflora* × *P. officinalis* are quite different. Eighteen of them had sepals more nearly resembling those of *P. officinalis* while sixteen were nearer to *P. albiflora* in this character. These results suggest that the genome of *P. officinalis*, in contrast to those of the tetraploids mentioned above, is heterozygous for the sepal character, and that segregation has occurred. This indicates a difference between the two original diploid genomes of this species. The importance of this fact in connection with the origin of *P. officinalis* and its relatives will be emphasized in a later publication.

The number of flowers per plant. The more primitive species of Paeonia—*P. Delavayi*, *P. Emodi*, most varieties of *P. anomala*, and *P. albiflora*—produce, in addition to the large bud that terminates the main axis, one to four small side branches that bear smaller buds and flowers. The other species, more advanced in their floral characteristics, bear a single flower on each stem.

Table 3 shows the inheritance of this character in interspecific crosses. A + indicates the presence of side buds, a —, their absence. A (+) in-

dicates that the side branches are weakly developed, and frequently fail to produce flowers.

This table shows that, as in the case of sepal characteristics, the hybrids resemble more nearly the primitive parent, although here exceptions are observed. One among the diploid species is the hybrid *P. anomala Veitchii* \times *P. triternata Mlokoewitschii*. Its significance is reduced, however, by the fact that side branches are often weakly developed or absent in

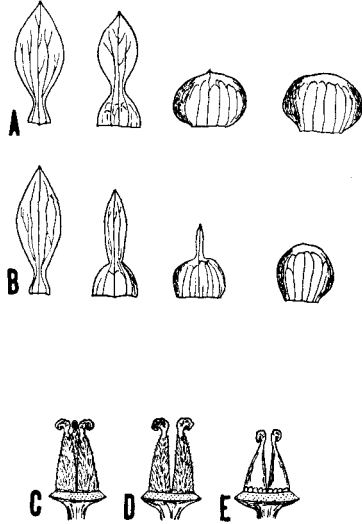


FIGURE 3.—The sepals of: A. *Paonia tomentosa*; B. *P. albiflora* \times *tomentosa*, "Seraph." The gynaecia, at anthesis, of C. *P. tomentosa*; D. *P. albiflora* \times *tomentosa*, "Seraph"; E. *P. albiflora* (Vilmorin seedling). A, B, $\times \frac{1}{2}$, C-E $\times 1$.

Veitchii, while in its close relative, typical *P. anomala*, they are absent altogether.

In the triploid hybrids, as shown in the table, the expression of this character varies considerably, and no satisfactory explanation for its mode of inheritance was obtained.

The character of the seedlings. *Paonia tenuifolia* differs from all other species of the genus, except *P. Brownii*, in that the cotyledons of the young seedlings are regularly raised above the ground, becoming green and functional as in most dicotyledons, whereas in the other species (except rarely in the subgenus Moutan) they are retained within the seed as storage organs, and the first visible leaf of the young seedling is developed from the plumule. In all of the hybrids of which *P. tenuifolia* was the ovulate parent the cotyledons were raised in the seedlings, and this was true also of *P. anomala Veitchii* \times *P. tenuifolia* and *Woodwardii* \times *P. tenuifolia*. The retention of the cotyledons underground must be considered a specialized characteristic; hence in this as in the previous

cases the more primitive characteristic tends to be dominant in interspecific hybrids.

The leaf characteristics. In their leaf characteristics the hybrids are as a rule intermediate between their parents, and there is no apparent tendency for the primitive leaf, the much lobed but not divided type, to be dominant.

THE INHERITANCE OF INTERSPECIFIC DIFFERENCES

In interspecific hybridization, the usual result is that the F_1 hybrid is in every respect nearly or quite intermediate between its parents. EAST (1935) after an able summary of the characteristics of the numerous and well known interspecific hybrids in *Nicotiana* has concluded that the form of any organ in a species is the result of the action of a large number of genes working together to create a typical "reaction pattern," and that "all constituents of the pattern are active in modifying the pattern of the hybrids, and the grade of these modifications is little affected by differences in the amount of chromatin contributed" (p. 433).

The interspecific hybrids of *Paeonia* follow, in general, this principle. Intermediate "reaction patterns" similar to those figured by EAST occur regularly in hybrids between the diploid species in the following characteristics: leaf shape, lobing, and dissection; petal size and shape; shape and degree of development of the staminodial disk; and the shape of the follicles and stigmas. Nevertheless, the following exceptions may be noted.

1. In three characteristics, the reaction patterns of diploid hybrids tended to resemble more closely one parent than the other. These characteristics, sepal number and shape, the presence or absence of a branched inflorescence, and the character of the cotyledons in the seedling, are all of phylogenetic significance in the evolution of the genus, and in every case there is a tendency for the more primitive condition to be dominant over the more specialized or advanced. This is most striking in the case of the primitive nature of the cotyledons of the hybrids involving *P. tenuifolia* since here in some cases a gene introduced through the chromosomes of the pollen parent expresses itself at the earliest stage of the germination of the seed. The evidence from *Paeonia*, therefore, points to the fact that in certain characteristics involving the phylogenetic principle of reduction, most of the mutations which produce the difference between species are of the recessive type. A similar situation was found by CLAUSEN (1931) in *Viola*, where such primitive characteristics as the perennial habit are dominant in interspecific hybrids.

2. In one of the diploid-tetraploid crosses, *P. albiflora* \times *tomentosa*, the evidence points definitely to the fact that the tetraploid contributes more to the "reaction pattern" of the hybrid than the diploid. The greater resemblance to *P. tomentosa* in sepal characteristics has been described

above (table 2, fig. 3) and the resemblance of the F_1 hybrid to *P. tomentosa* in stature and leaf shape has been described by the senior author in a previous paper (SAUNDERS 1933b). There is an even more striking resemblance to *P. tomentosa* in the appearance of the follicles at anthesis, (fig. 3, C-E).

The triploid hybrids of another cross, *P. albiflora* \times *officinalis* did not show a striking resemblance to their tetraploid parent, but were nearly intermediate. This has been noted above for the sepal characteristics, and by SAUNDERS (1933b) for their stature. An analysis of leaf patterns was not feasible, due to the extreme complexity and variability of this character in *P. officinalis*, but the type of lobing characteristic of *P. albiflora* was very nearly approached in some of the hybrids. The explanation of the different results in these two sets of diploid tetraploid crosses probably lies in the different origin of the tetraploid parent. *P. tomentosa* is taxonomically a more homogeneous, constant species than *P. officinalis*, and the high number of multivalents found at meiosis in this species (to be described in a later paper), indicates that the two diploid genomes that compose its set are closely homologous, that is, it is genetically an autopolyploid. *P. officinalis* is, however, highly polymorphic, has a low proportion of multivalents at meiosis, and the systematic and genetic evidence points to its allotetraploid nature. Furthermore the diploid genomes composing it were probably derived from widely separated diploid species, which differed profoundly in leaf, sepal, and follicle characteristics. In this connection it is perhaps significant that all three of the *Nicotiana* tetraploids referred to by EAST are probably allotetraploids (GOODSPEED 1934), and that at least one, *N. Tabacum*, is derived from two widely different diploid species. The evidence here presented, therefore indicates that while, as EAST maintains, the *amount* of chromatin contributed by one parent has little effect on the genic pattern of the hybrid, nevertheless the number of duplicated gene pairs contributed by (that is, the amount of autopolyploidy in) one parent is of significance. The same conclusion was reached by ANDERSON (1936) after a comparison of diploid and tetraploid hybrids of *Tradescantia canaliculata* and *T. subaspera*.

SOME ABNORMAL CHARACTERISTICS OF THE HYBRIDS

A discussion of the appearance of these hybrids is not complete without an account of the striking abnormalities that have appeared in some of them. These are:

Abnormal growth of the petals with partial suppression of the stamens. In the hybrid, *P. triternata Mlokoewitschii* \times *P. anomala Woodwardii* the young buds are abnormal in their oblong rather than spherical shape. This is due to the premature elongation of the sepals, and the failure of the

petals to develop at a normal rate. The buds often do not open completely, expanding only enough so that the color of the petals may be seen. At this time the petals have reached normal size, being intermediate between those of the parents of this hybrid. The stamens are almost completely suppressed, appearing as strap-like appendages barely 2.5 mm long, as opposed to 10 mm and 12 mm in the two parents (fig. 4C). In the central flower, these rudiments never produce pollen mother cells, but the lateral flowers contain a few scattered, minute anthers in which are formed pollen mother cells, and, after a very irregular meiosis, a scant amount of sterile pollen.

In the hybrid between *Mlokosewitschii* and *P. anomala*, the young buds are elongate like those of *Mlokosewitschii* × *Woodwardii*, but they finally

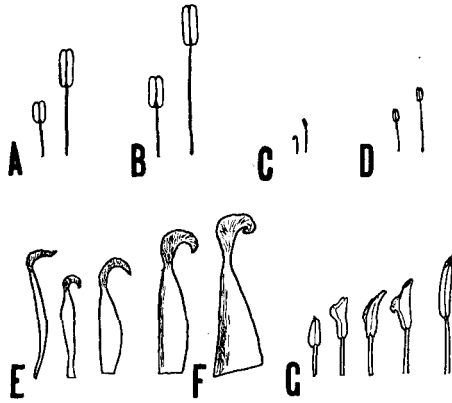


FIGURE 4.—The stamens of: A. *Paeonia anomala Woodwardii*; B. *P. triternata Mlokosewitschii*; C. *Mlokosewitschii* ♀ × *Woodwardii* ♂; D. *Mlokosewitschii* ♀ × *P. anomala typica* ♂. All × 2. E. Modified stamens of *Paeonia albiflora* × *P. anomala Woodwardii*; F. Normal follicle of the same (at anthesis); G. Modified stamens of *P. albiflora* × *P. anomala Veitchii*. All × 2.

expand into normal flowers. The anthers are somewhat suppressed, being only one sixth to one fifth as long as those of the parents, but they produce some, though a reduced amount, of pollen (fig. 4D).

The third hybrid of this series was a reciprocal to the others, *P. anomala Veitchii* × *P. triternata Mlokosewitschii*. It is perfectly normal in bud shape, and in the development of its sepals, petals, and stamens. It produces as much pollen as its parents, although this is almost completely sterile, due to the very abnormal meiosis, which will be described in a subsequent paper. Hence it seems likely that the suppression of growth of the organs appears only when *Mlokosewitschii* is the ovulate parent for this series of crosses.

Partial sex reversal. An even more striking abnormality appeared in the crosses between *P. albiflora*, and the various varieties of *P. anomala*. In the hybrids *P. albiflora* × *P. anomala Woodwardii*, and *P. albiflora* × *P.*

anomala Beresowskii, the stamens are replaced by a large number of rudimentary carpels. These have normal looking stigmas, but they are completely open along the ventral suture, and do not produce even the rudiments of ovules (fig. 4E, 4F). The abnormality is most pronounced in the central flower. In the lateral flowers stamens with thin, strap-shaped anthers are often formed, which, in *P. albiflora* × *anomala typica* occasionally produce pollen. The true follicles are normal in appearance, though in some cases more numerous than in normal flowers, and occasionally develop seed. In the final hybrid of this series, *P. albiflora* × *P. anomala Veitchii*, the abnormality is much less pronounced. Here stamens with anthers are regularly produced, but these have pink, sterile tips, and occasionally produce stigmas from their tips or sides (fig. 4G). No reciprocal hybrids of this series have yet been made, so that there is no evidence to indicate whether or not these abnormalities would, as in the previous case, be absent when *P. anomala* and its relatives were used as the ovulate parents.

INHIBITION OF GROWTH IN RECIPROCAL HYBRIDS

The abnormalities found in the hybrids between *P. triternata Mlokosewitschii* and the varieties of *P. anomala* may be compared, so far as our results go, with the situation found by many workers in interspecific hybrids of *Epilobium* (LEHMANN 1928, 1931; MICHAELIS 1931 etc.). The parallelism between the two cases may be summarized as follows:

1. In both cases, the abnormalities found in the F_1 hybrids consist exclusively in the inhibition of growth or development in one or more organs or (in the case of *Epilobium*) in the plant as a whole.

2. In both cases there is a reciprocal difference. In *Epilobium* the inhibitions appear most abundantly in the plasma of *E. hirsutum* and *E. parviflorum*, when the pollen parent is a species widely separated taxonomically, while in our case the abnormalities appear under similar conditions in the plasma of *P. triternata Mlokosewitschii*.

3. In both cases the amount of inhibition varies with the genetic constitution of the pollen. SCHWEMMLE (1934) has demonstrated the presence of Mendelian factors in races of *E. roseum* which affect the intensity of inhibition in the plasma of *E. hirsutum*. In *Paeonia*, the inhibition in growth of the anthers is much more marked in *P. triternata Mlokosewitschii* × *P. anomala Woodwardii* than in *Mlokosewitschii* × *P. anomala typica*. In this case there is an interesting correlation between the extent of the inhibition and the luxuriance of the plant. The variety *Woodwardii* has small leaves and flowers; the typical form is taller, and with larger leaves and flowers. It is likely, therefore, that the genic complex in *Woodwardii* which produces the strong growth inhibitions in the plasma of *Mlokosewitschii* is

the same or similar to that which effects the reduction in size of the organs of the homozygote. The stamens of *Woodwardii* are normally smaller than those of the typical form, the average lengths being 9 and 11 mm respectively, but in their hybrids in the plasma of *Mlokošewitschii* the difference is much greater, the average lengths being 2 mm and 7 mm respectively.

The situation in *Paeonia* is also closely parallel to that found by DOBZHANSKY (1935) and DOBZHANSKY and STURTEVANT (1935) in *Drosophila pseudoobscura*. These workers have demonstrated by means of carefully controlled genetical tests that a reciprocal difference in testis size found in crosses between race A and race B of this species is due unquestionably to maternal effect rather than to plasmatic inheritance. In view of their findings, and the similar though less complete results of SCHWEMMLE (1934) in *Epilobium* the writers are inclined to believe that a similar explanation is the most satisfactory one for the phenomena described in *Paeonia*, as well as in *Epilobium*, in spite of a considerable amount of evidence to the contrary in the latter case (MICHAELIS 1933).

As a possible clue to the explanation of this maternal effect the experiments of DELLINGSHAUSEN (1935) which demonstrated a greater viscosity in the cytoplasm of *Epilobium hirsutum* than in that of *E. luteum*, and a correspondingly lower permeability to electrolytes and lipid soluble substances, are worth noting. In the living sporocytes of *Paeonia triternata* and its subspecies the cytoplasm is much more opaque and more heavily granular than that of *P. anomala*, and that of the former species stains more heavily after fixation. This suggests that the cytoplasm of *P. triternata* has, like that of *E. hirsutum*, a high viscosity and low permeability, and that this is directly connected with the maternal effect. Apparently cytoplasm of a high viscosity and a low permeability to lipid soluble substances is most favorable to the action of growth inhibiting genes in the hybrid genom, while a less viscous and more permeable cytoplasm favors the action of the genes determining normal growth.

The case of partial sex reversal parallels that of the inhibition of anther growth in the following respects:

1. Here also the growth of the anthers is inhibited. When not replaced by rudimentary carpels, they are usually thin, strap-shaped structures which produce very little or no pollen.

2. The abnormalities appeared only when *P. anomala* and its subspecies were used as the pollen parents, although the ovulate parent was in this case *P. albiflora*, which is a little less distant taxonomically from *P. anomala* than is *P. triternata Mlokošewitschii* and which does not have the dense appearing cytoplasm of that species.

3. The different varieties of *P. anomala*, when used as pollen parents, produce the same difference in the degree of expression of the character in

the F_1 hybrids. In the F_1 of *P. albiflora* ♀ × *P. anomala Woodwardii* ♂ or *Beresowskii* ♂ (a plant similar in the size of its leaves and flowers to *Woodwardii*) it is most extreme. In *P. albiflora* × *P. anomala typica* it is less so; while in *P. albiflora* × *P. anomala Veitchii*, which has leaves and flowers as large as or larger than those of subsp. *typica*, in the F_1 hybrid it was the least noticeable.

4. In both cases the character is expressed most strongly in the central and less so in the lateral flowers.

In plants, sex reversal can be caused either by the environment (*Cannabis*, SCHAFFNER 1921), or by the action of combinations of genes (*Zea*, JONES 1934; *Nicotiana*, GOODSPEED 1930). Neither of these agencies can explain satisfactorily the present case. The hybrid plants have been growing for several years in the same plot with normal species and hybrids, and have year after year produced unfailingly these abnormalities. Hence the action of the environment can in this case be dismissed without question. On the other hand, no similar sex reversals have appeared in any of the parent species, or more particularly in any of the numerous horticultural varieties of *P. albiflora* which have been grown in this garden, nor in any interspecific hybrids except those mentioned above. Hence any explanation based purely on genic action must assume that there are certain genes and modifiers in *P. albiflora* and *P. anomala* which acting separately have no effect on sex expression, but, acting together in this particular set of hybrids, produced the results observed. Whether this is the correct explanation of these sex reversals, or whether they are due to a cytoplasmic effect similar to that responsible for the inhibitions in the *Mlokosewitschii* × *anomala* hybrids, will depend on the nature of the reciprocal hybrid, when or if it is obtained.

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SUMMARY

1. Some of the results of 20 years of hybridization work with wild species and cultivated forms of *Paeonia* are described.

2. The degree of compatibility between diploid species is a valuable criterion for delimiting species and subgenera, but gives no information concerning the interrelationships between the different species of a subgenus.

3. Tetraploid species are as a rule completely compatible with each other.

4. Of the diploid species, *P. albiflora* can be crossed relatively easily with any of the tetraploid species, but the others cross with difficulty or

not at all. The cross *P. albiflora* × *tomentosa* is made most easily with the tetraploid as pollen parent, while in the cross *P. albiflora* × *officinalis* there is no reciprocal difference in compatibility.

5. The F₁ hybrids are morphologically intermediate between their parents, and in crosses involving *P. albiflora* or *P. officinalis* show a remarkable degree of variability.

6. In some characteristics, namely, the number and morphology of the sepals, the presence or absence of side buds, and the character of the cotyledons, there is a definite tendency for dominance of those traits which are less specialized, and therefore phylogenetically primitive.

7. In diploid-tetraploid crosses, there is a strong tendency for the dominance of the characteristics of the tetraploid, if the latter is, as indicated by its morphological and cytological characteristics, nearly or completely of autopolyploid origin.

8. In F₁ hybrids between *P. triternata Mlokoewitschii* and the various varieties of *P. anomala*, there is a marked suppression of the anthers when the former is the ovulate parent, but one series of hybrids with it as the pollen parent is normal in this respect. This suggests the presence of a plasmatic inhibition of anther development, which varies in intensity depending on the subspecies of *P. anomala* used.

9. In F₁ hybrids between the various subspecies of *P. anomala* and *P. albiflora*, when the latter is the ovulate parent, varying degrees of sex reversal of the anthers are found, ranging from the presence of stigmatoid tips on the anthers of *P. albiflora* × *anomala Veitchii* to complete replacement of the anthers by rudimentary follicles in *P. albiflora* × *anomala Woodwardii*. Since there is a marked correlation between the degree of effectiveness of the different varieties of *P. anomala* in producing this abnormality when used as pollen parents on *P. albiflora*, and their effectiveness in producing suppression of the anthers when used on *P. triternata Mlokoewitschii*, it is suggested that the two phenomena are similar in origin.

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