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*Aquilegia***



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INTERSPECIFIC HYBRIDIZATION AND ITS EVOLUTIONARY SIGNIFICANCE IN THE GENUS *AQUILEGIA*¹

RONALD J. TAYLOR

Taylor, R. J. (Western Washington State College, Bellingham, Washington) Interspecific hybridization and its evolutionary significance in the genus *Aquilegia*. *Brittonia* **19**: 374–390. 1967.—Various hybrid combinations of 18 species and varieties of *Aquilegia* were produced, and several cross-pollinations were made which failed to produce hybrids. Pollen fertility of hybrids was generally high, with a total mean of approximately 50%, but highly variable among hybrid combinations and consistently lower than that of parental species. With the exception of frequent occurrence of giant pollen grains and rare synaptic failure of one or two homologous pairs, meiotic behavior in hybrid pollen mother cells appeared to be completely regular.

Representatives of the *Vulgaris* Complex were observed to be intermediate genetically and morphologically leading to the interpretation of ancestral status among extant species. The greatest genetic diversity within the genus was shown to exist between *A. ecalcarata* and members of the *Caerulea* Complex.

The genus *Aquilegia* (Ranunculaceae) consists of 67 species (Munz, 1946) of perennial herbs, widely distributed in temperate and mountainous regions of the northern hemisphere. The most important of relatively recent systematic treatments of the genus are those by Payson (1918) on North American species; by Boothman (1934), a brief treatment in which the author attempts to rank the species phylogenetically; and by Munz (1946), a monograph of *Aquilegia* which includes the first key to the species of the genus as a whole.

The species of *Aquilegia* are, in general, very closely related genetically as has been indicated by extensive hybridization studies (Winge, 1928; Anderson & Schafer, 1931; Kappert, 1943; Linnert, 1961; Prazmo, 1965a, 1965b). The barriers which separate the species are more strongly geographic than cytogenetic. However, differentiation of the genus has progressed a great distance morphologically with considerable variation in configuration, size, and color of flowers and to a lesser degree in gross habit of the plants. Because of its cytological uniformity and consequent crossability, the genus was referred to as "one huge cenospecies" by Clausen, Keck and Hiesey (1945), a concept which generally has been accepted by subsequent authors.

In the genus various groups of species or species complexes have been distinguished on the basis of assumed relationships. Payson (1918) proposed such a classification dealing with the North American species. Boothman (1934) proposed a similar grouping, selecting representative species from the entire range of the genus. Grant (1952) arranged the species in accordance with flower structure and consequent pollinating mechanism. Prazmo (1965b) distinguished between species complexes on the basis of morphological differences, crossability, and hybrid fertility. Inasmuch as there is an overlap of botanical characters among taxa of different complexes, such a classification scheme lacks definiteness. It does, however, tend to group the species phylogenetically. In this treatment the following complexes are recognized: *Vulgaris*, *Ecalcarata*, *Alpina*, *Viridiflora*, *Flabellata*, *Brevistyla*, *Caerulea*, and *Canadensis*.

The present study was designed to supplement existing knowledge regarding phylogenetic relationships within the genus. The species used here were selected on the

¹This paper is adapted from a thesis submitted to Washington State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The advice and direction of Dr. Marion Ownbey, Professor of Botany and Genetics, is gratefully acknowledged.

TABLE I
SPECIES AND THEIR DISTRIBUTION

Name of species	Complex	Distribution
<i>A. atrata</i> Kock	Vulgaris	Bavarian Alps
<i>A. olympica</i> Boiss.	Vulgaris	Caucasus of S. Russia, Turkey, and Iran
<i>A. oxysepala</i> var. <i>yabeana</i> (Kitagawa) Munz	Vulgaris	Himalayas of China
<i>A. vulgaris</i> L.	Vulgaris	Eurasia
<i>A. ecalcarata</i> Maxim.	Ecalcarata	Himalayas of China
<i>A. alpina</i> L.	Alpina	Northwestern Europe
<i>A. viridiflora</i> Pall.	Viridiflora	Altai and Himalayan Mountains of Mongolia and China
<i>A. flabellata</i> Sieb.	Flabellata	Japan
<i>A. sibirica</i> Lam.	Flabellata	Siberia and Northern Mongolia
<i>A. brevistyla</i> Hook.	Brevistyla	Northwestern North America
<i>A. canadensis</i> L.	Canadensis	Eastern North America
<i>A. flavescens</i> Wats.	Canadensis	Rocky Mountains of North America
<i>A. shockleyi</i> Eastwood	Canadensis	Southwestern United States
<i>A. skinneri</i> Hook.	Canadensis	Northern Mexico
<i>A. caerulea</i> James	Caerulea	Rocky Mountains of North America
<i>A. longissima</i> Gray	Caerulea	Southwestern United States

basis of their geographical distribution and general morphology, i.e., the genus was sampled in such a way as to obtain maximum geographic coverage and extensive morphologic variation.

MATERIALS AND METHODS

Establishment of Parental Species

The seeds of *Aquilegia* used in the present investigation were secured primarily through international seed exchanges. The original source of most of them is unknown.

In September, 1961, the seeds of several species were planted in flats in the greenhouse and the seedlings were allowed to grow throughout the winter months. In March, after the plants had become rather large, the flats were placed outside, causing the seedlings to enter into a state of dormancy. In the latter part of April, after the weather had warmed sufficiently to stimulate spring growth, 12 seedlings of each species were transplanted into the experimental garden. The vernalization treatment was effective and the plants, with few exceptions, flowered the first year.

At the time of anthesis, identity of all plants was checked critically using the monograph of Munz (1946). If the plants did not fit the description of the species under which name the seeds were obtained, or if variability among individual plants was excessive, they were discarded. Other tests made to assure purity of species were pollen fertility counts and analyses of meiotic regularity. The procedures followed in making these tests will be discussed under subsequent headings. Table I is a list of species used in this study.

Techniques of Hybridization

All crosses were made under controlled conditions, usually reciprocally and in duplicate. The pollinations were effected twice. If seeds were not obtained from the first two pollinations, additional crosses were made. Table II provides a complete record of attempted crosses and is a partial index to the degree of crossability based on pollen stainability.

The seeds produced through hybridization were collected as they matured and planted in the greenhouse soon thereafter. The hybrid seedlings were processed in the same manner as were the parental species.

TABLE II
RECORD OF HYBRIDIZATION AND POLLEN FERTILITY OF F₁ HYBRIDS*

♀ ↓	♂→	alp	atr	brev	caer	can	ecal	flab alba	flav	long	oly	oxy	sho	sib	skin	vir	vul
<i>alpina</i>	—	95	68	—	—	70	—	—	69	75	—	58	—	83	—	0	—
<i>atrata</i>	+	—	42	58	48	0	—	—	—	59	91	73	59	—	—	74	+
<i>brevistyla</i>	51	—	—	—	—	43	—	54	69	81	—	0	—	—	—	42	—
<i>caerulea</i>	—	+	89	—	+	0	—	—	—	95	60	0	+	74	—	64	—
<i>canadensis</i>	—	—	+	—	—	—	—	—	—	—	—	0	—	0	—	0	—
<i>ecalcarata</i>	0	77	0	0	0	—	+	0	40	0	—	68	0	—	0	61	—
<i>flabellata</i>	—	—	—	—	28	—	—	—	61	—	—	—	—	—	—	45	—
<i>flabellata</i> var. <i>alba</i>	68	—	—	—	36	—	—	—	17	—	—	< 1	—	—	—	65	—
<i>flavescens</i>	—	—	+	+	+	0	—	65	—	+	0	0	—	—	—	0	—
<i>longissima</i>	+	53	84	93	+	0	—	—	+	—	39	0	91	61	64	13	55
<i>olympica</i>	+	+	0	59	—	61	—	—	+	40	—	—	—	—	—	54	—
<i>oxysepala</i> var. <i>yabeana</i>	—	—	—	—	0	67	—	—	0	0	0	—	0	—	0	0	0
<i>shockleyi</i>	75	17	+	+	+	41	—	—	—	+	57	0	—	—	93	—	67
<i>sibirica</i>	+	80	0	60	—	64	—	—	57	+	—	84	—	—	—	62	+
<i>skinneri</i>	—	59	—	—	—	0	—	—	—	—	59	0	—	—	—	0	—
<i>viridiflora</i>	0	70	0	67	0	63	58	65	42	0	57	57	65	61	0	—	61
<i>vulgaris</i>	96	+	—	70	—	79	—	—	—	0	—	73	60	—	62	56	—

* The numbers represent the mean pollen fertility of the respective hybrid progeny. "0" indicates only that pollination was effected and mature F₁ hybrids were not obtained. (For a further analysis of unsuccessful cross-pollinations see Table III). The symbol "—" indicates that the cross was not made, and "+" indicates cross-pollination followed by the production of seeds which were not planted.

Chromosomal Studies

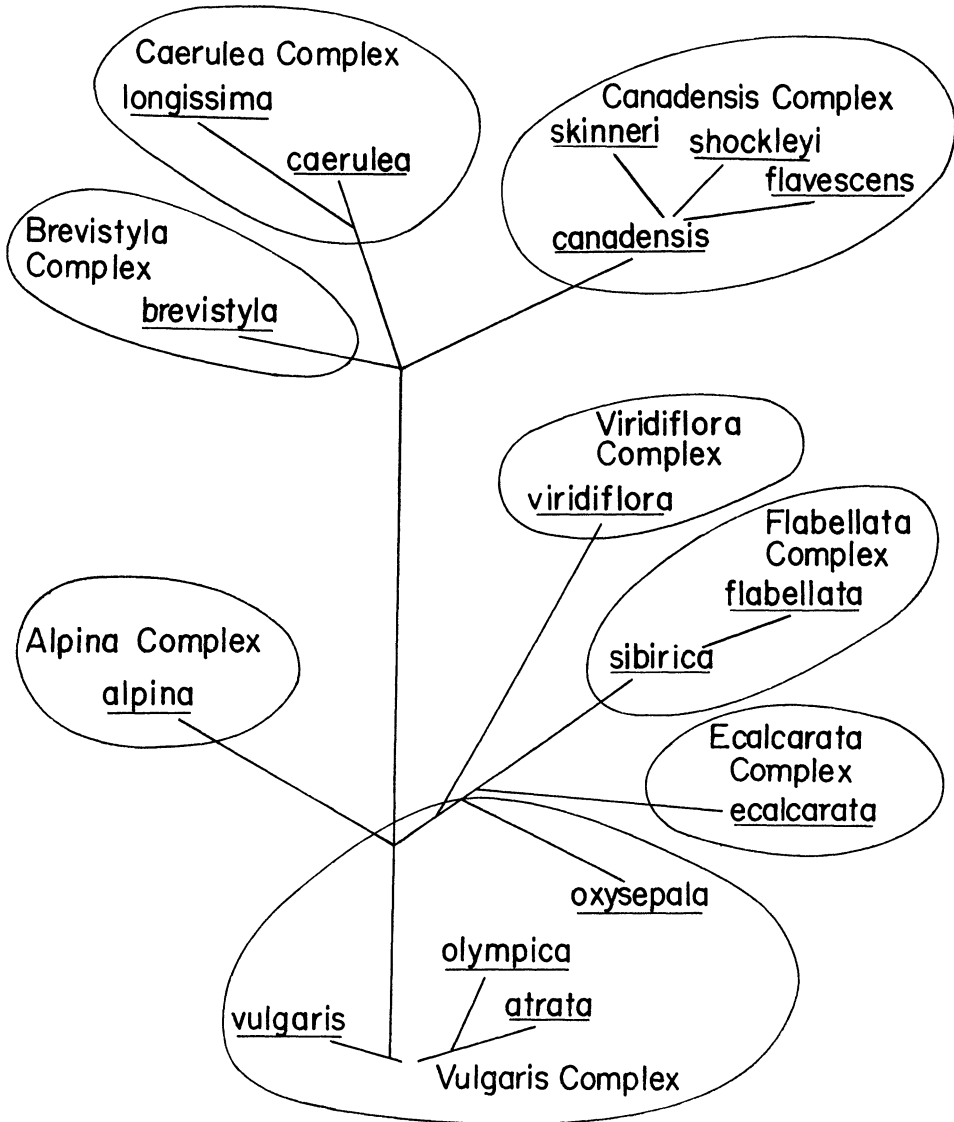
For the study of meiotic divisions, acetocarmine squash techniques of pollen mother cells were employed. Slides prepared in this way were allowed to dry for approximately 45 min to remove excess stain after which time the cover slips were ringed with Massart's solution² making them semipermanent.

Fertility Determinations

Pollen fertility: The distinction between empty, sterile pollen grains and well filled, presumably fertile ones is usually clear in *Aquilegia*, and this distinction is enhanced by acetocarmine staining. However, while there can be no doubt that the small, empty pollen grains are sterile, there is no assurance that all of the well filled grains are capable of germination. The difficulty of arriving at an exact fertility determination is increased in *Aquilegia* by the frequent presence of pollen grains intermediate in size and stainability. Therefore, the percentages given in this treatment may not always be exact indices of pollen fertility, but are very useful on a comparative basis. Intermediate size pollen grains, however, were rarely observed in parental species or fertile hybrids; therefore, their presence was associated with a high degree of sterility.

A minimum count of 500 pollen grains including fertile and sterile grains was made from each plant, and from these figures, the percentage of fertility was calculated. With few exceptions, counts were made from every plant which flowered.

² Massart's solution consists of 50 grams of gum arabic, 100 grams of chloral hydrate, 16 cc of glycerin, and 100 cc of water.

FIG. 1. Phylogeny of *Aquilegia*.

Seed germination: In most cases, seeds which appeared to have developed normally germinated readily, but some did not. In preliminary experiments it was established that seeds of some species remained dormant much longer than those of others. This trait could be inherited by the hybrids, and failure of seed germination under standard conditions would not necessarily imply lack of viability. In some hybrid groups, the tetrazolium chloride test (Machlis & Torrey, 1956) was used to indicate the viability of seeds which otherwise appeared normal but had failed to germinate.

In certain crosses where seed production did not follow pollination, the pollen tube squash technique was employed to determine if pollen germination and pollen tube penetration had taken place. In accordance with widely used techniques, the styles were removed from the flower 2-3 hr after pollination, squashed between glass slides,

fixed in 70% alcohol for 2 min, and stained in a solution of 1% iodine plus 1.5% potassium iodide in 30% alcohol. Pollen tube penetration was then determined by microscopic examination.

Voucher Materials

Pressed specimens of all hybrid combinations and of most species are filed in the Washington State University Herbarium (WS). In addition, black and white and colored pictures were taken of most experimental plants and have been retained by me.

RESULTS

Analysis of Meiosis

Aquilegia is not a favorable genus for cytological study. The chromosomes are small, the meiotic karyotypes of different species are very similar, and the seven meiotic bivalents are uniform in size and configuration thus lacking individuality.

Meiotic chromosomes from several cells of each species and hybrid were examined. With very few exceptions, meiosis was found to be regular in all cells which could be analyzed. Observations on meiotic behavior of F_1 interspecific hybrids are presented in Table III. Chiasmata frequency was consistently low in metaphase bivalents. In no cases were bivalents observed to have more than two chiasmata, and in most instances they contained a single, terminal chiasma. The chromosome number of all species examined was $n = 7$.

Pollen Fertility and Analysis

Pollen fertility in hybrid Columbines as a whole is much less uniform than is the ability to form regular pairs of chromosomes at meiosis, or to produce viable seeds. While the percentage of fertility among members of full sib groups was, in general, uniform, the variation among groups ranged from 0% fertility in some to nearly 100% in others. Table II facilitates a direct comparison of hybrid fertility.

The average pollen fertility of the species was approximately 90%, ranging from 78.7 ± 5.4 in *Aquilegia ecalcarata* to 98.2 ± 1.0 in *A. caerulea*. That of the hybrids was slightly above 50%. In only a few instances did the fertility of the hybrids closely approach that of the parental species, indicating that in most cases genetic differentiation affecting fertility had taken place. However, lack of crossability or low pollen fertility may not be caused by extensive genotypic differentiation, but may result from single or few genic changes. This is exemplified by the inconsistency of behavior of some closely related taxa, i.e., *A. canadensis*, *A. skinneri*, and *A. shockleyi*, when in hybrid combination with a fourth species.

Pollen grains of intermediate size and stainability were common or even predominant in certain hybrid combinations. In these instances, the fertility counts became highly subjective inasmuch as the line between fertile and sterile grains was drawn arbitrarily. The deviation from actual fertility percentages, however, was probably in the direction of a higher rather than a lower score. Another pollen abnormality frequently observed in some hybrid combinations was the presence of apparently viable grains of giant size, diploid or even tetraploid.

Inheritance Patterns

In this study there has been no attempt to distinguish between multiple or single genetic factor inheritance expressed in morphological traits. The present data were obtained only from F_1 hybrids, and second generation segregation ratios are unknown. However, as shown by the variability of expression in the F_1 's, such traits as floral color and spur length are not generally controlled by a single pair of genes acting in a Mendelian manner.

TABLE III
 POLLEN STABILITY, MEIOTIC BEHAVIOR, OR POINT OF HYBRID FAILURE

Hybrid combinations	Mean percent and standard error of stainable pollen	Observations of meiotic behavior or point of hybrid failure
<i>alpina</i> × <i>atrata</i>	95 ± 2.1	Metaphase I cells showed no meiotic irregularities. Anaphase cells were not observed
<i>alpina</i> × <i>brevistyla</i>	68 ± 5.4 (two giant pollen grains were observed)	Univalents were observed in a comparatively few metaphase cells but were properly oriented indicating precocious separation
<i>brevistyla</i> × <i>alpina</i>	51 ± 6.5	Meiosis appeared regular although material examined was barely satisfactory
<i>alpina</i> × <i>ecalcarata</i>	70 ± 2.8	Metaphase cells were not observed but anaphase I cells showed regular segregation
<i>ecalcarata</i> × <i>alpina</i>	—	Seeds failed to germinate
<i>flabellata</i> var. <i>alba</i> × <i>alpina</i>	68 ± 4.1	Meiosis appeared regular in all stages of division
<i>alpina</i> × <i>flavescens</i>	69 ± 3.9	Meiosis appeared regular in all stages of division
<i>alpina</i> × <i>longissima</i>	75 ± 4.0	Meiosis appeared regular in all stages of division
<i>longissima</i> × <i>alpina</i>	no counts	Meiosis appeared regular in all stages of division
<i>alpina</i> × <i>oxysepala</i> var. <i>yabeana</i>	58 ± 6.0	Meiotic preparations were unsatisfactory
<i>shockleyi</i> × <i>alpina</i>	75 ± 4.8	No satisfactory anaphase cells were observed; occasionally, late metaphase cells contained a pair of univalents
<i>alpina</i> × <i>sibirica</i>	82 ± 4.6	Meiosis appeared regular in all stages of division
<i>alpina</i> × <i>viridiflora</i>	—	No apparent fertilization
<i>viridiflora</i> × <i>alpina</i>	—	Fertilization occurred but seeds aborted prior to maturity
<i>vulgaris</i> × <i>alpina</i>	96 ± 2.3	Meiosis appeared regular in all stages of division
<i>atrata</i> × <i>brevistyla</i> (a single plant)	42	No meiotic analyses were made
<i>atrata</i> × <i>caerulea</i>	58 ± 4.2	Meiosis appeared regular in all stages of division
<i>atrata</i> × <i>canadensis</i> (four plants)	47 (several giant pollen grains were observed)	Two metaphase cells possessed a pair of univalents, segregation appeared regular in all anaphase cells observed

TABLE III. Continued.

Hybrid combinations	Mean percent and standard error of stainable pollen	Observations of meiotic behavior or point of hybrid failure
<i>atrata</i> × <i>ecalcarata</i>	—	Seeds were produced but failed to germinate
<i>ecalcarata</i> × <i>atrata</i> (a single plant)	77	A few metaphase cells contained a pair of univalents; anaphase appeared regular
<i>atrata</i> × <i>longissima</i>	59 ± 5.8 (numerous giant pollen grains were observed)	Metaphase I cells showed no meiotic irregularities; one anaphase I cell had 6 chromosomes at one pole and 8 at the other. One anaphase cell had what appeared to be a bridge and a fragment
<i>longissima</i> × <i>atrata</i>	53 ± 4.0	Meiotic material was unsatisfactory
<i>atrata</i> × <i>olympica</i>	91 ± 2.2	Meiosis appeared regular in all stages of division
<i>atrata</i> × <i>oxysepala</i> var. <i>yabeana</i>	73 ± 3.6	Meiotic material was barely satisfactory and chromosomes appeared "sticky"
<i>atrata</i> × <i>shockleyi</i>	60 ± 4.9	Meiosis appeared regular in all stages of division
<i>shockleyi</i> × <i>atrata</i>	17 ± 21.0 (the range was from 53% to nearly 0% fertility)	Meiosis appeared regular in all stages of division
<i>sibirica</i> × <i>atrata</i>	80 ± 4.0	Meiosis appeared regular in all stages of division
<i>skinneri</i> × <i>atrata</i>	59 ± 6.0	Meiosis appeared regular in all stages of division
<i>atrata</i> × <i>viridiflora</i>	74 ± 4.4	Meiotic material was unsatisfactory
<i>viridiflora</i> × <i>atrata</i>	70 ± 3.6	Meiosis appeared regular in all stages of division
<i>caerulea</i> × <i>brevistyla</i>	89 ± 2.7	Meiosis appeared regular in all stages of division
<i>brevistyla</i> × <i>ecalcarata</i> (two plants)	43	Meiosis appeared regular in all stages of division
<i>ecalcarata</i> × <i>brevistyla</i>	—	Seeds were produced but failed to germinate
<i>brevistyla</i> × <i>flabellata</i> var. <i>alba</i>	54 ± 3.6	Meiotic material was barely satisfactory but meiosis appeared to be regular
<i>brevistyla</i> × <i>flavescens</i>	69 ± 3.8	Meiosis appeared regular in all stages of division
<i>brevistyla</i> × <i>longissima</i>	81 ± 4.1	Meiosis appeared regular in all stages of division

TABLE III. Continued.

Hybrid combinations	Mean percent and standard error of stainable pollen	Observations of meiotic behavior or point of hybrid failure
<i>longissima</i> × <i>brevistyla</i>	84 ± 4.3	Meiosis appeared regular in all stages of division
<i>olympica</i> × <i>brevistyla</i>	—	Seeds were produced but failed to germinate
<i>brevistyla</i> × <i>oxysepala</i> var. <i>yabeana</i>	—	Seeds were produced but failed to germinate*
<i>sibirica</i> × <i>brevistyla</i>	—	Hybrids did not flower the first year
<i>brevistyla</i> × <i>viridiflora</i>	42 ± 5.3 (numerous giant pollen grains were observed)	One anaphase I cell showed unequal chromosomal segregation (6 + 8); all other cells appeared regular
<i>viridiflora</i> × <i>brevistyla</i>	—	Fertilization occurred but seeds aborted prior to maturity
<i>caerulea</i> × <i>ecalcarata</i>	—	Seeds were produced but failed to germinate*
<i>ecalcarata</i> × <i>caerulea</i> (four plants)	—	Seedlings were weak and died prior to maturity
<i>caerulea</i> × <i>longissima</i>	95 ± 2.8	Meiosis appeared regular in all stages of division
<i>longissima</i> × <i>caerulea</i>	93 ± 2.3	No meiotic analyses were made
<i>caerulea</i> × <i>olympica</i>	60 ± 3.8	Meiosis appeared regular in all stages of division
<i>olympica</i> × <i>caerulea</i>	59 ± 4.0	Meiosis appeared regular in all stages of division
<i>caerulea</i> × <i>oxysepala</i> var. <i>yabeana</i>	—	Seeds were produced but failed to germinate*
<i>caerulea</i> × <i>sibirica</i>	73 ± 4.9	Meiosis appeared regular in all stages of division
<i>sibirica</i> × <i>caerulea</i>	60 ± 4.2	No meiotic analyses were made
<i>caerulea</i> × <i>viridiflora</i>	64 ± 6.1 (several giant pollen grains were observed)	Meiosis appeared regular in all stages of division
<i>viridiflora</i> × <i>caerulea</i>	67 ± 3.6 (several giant pollen grains were observed)	Meiosis appeared regular in all stages of division
<i>vulgaris</i> × <i>caerulea</i>	70 ± 3.9	Meiosis appeared regular in all stages of division
<i>ecalcarata</i> × <i>canadensis</i>	—	Seeds were produced but failed to germinate*

* The tetrazolium chloride test was used to confirm lack of seed viability.

TABLE III. Continued.

Hybrid combinations	Mean percent and standard error of stainable pollen	Observations of meiotic behavior or point of hybrid failure
<i>flabellata</i> × <i>canadensis</i>	28 ± 5.8 (several giant pollen grains were observed)	Two anaphase I cells showed numerically unequal chromosomal segregation; metaphase I cells appeared regular
<i>flabellata</i> var. <i>alba</i> × <i>canadensis</i>	36 ± 7.1 (several giant pollen grains were observed)	Meiotic material was poor but showed no indication of irregularity
<i>canadensis</i> × <i>oxysepala</i> var. <i>yabeana</i>	—	Seeds were produced but failed to germinate*
<i>oxysepala</i> var. <i>yabeana</i> × <i>canadensis</i>	—	Fertilization occurred but seeds aborted prior to maturity
<i>canadensis</i> × <i>sibirica</i>	—	Seedlings were weak and did not flower
<i>canadensis</i> × <i>viridiflora</i>	—	Seedlings were weak and died
<i>viridiflora</i> × <i>canadensis</i>	—	Seedlings were weak and died
<i>ecalcarata</i> × <i>flabellata</i> var. <i>alba</i>	—	Six seedlings were produced but were weak and did not flower
<i>ecalcarata</i> × <i>flavescens</i> (four plants)	40 ± 5.9	Meiotic material was poor but showed no indication of irregularity
<i>flavescens</i> × <i>ecalcarata</i>	—	Fertilization did not occur
<i>ecalcarata</i> × <i>longissima</i>	—	Fertilization occurred but seeds aborted prior to maturity
<i>olympica</i> × <i>ecalcarata</i>	61 ± 4.1	Meiosis appeared regular in all stages of division
<i>longissima</i> × <i>ecalcarata</i>	—	Seeds were produced but failed to germinate*
<i>ecalcarata</i> × <i>oxysepala</i> var. <i>yabeana</i>	68 ± 3.2	Meiosis appeared regular in all stages of division
<i>oxysepala</i> var. <i>yabeana</i> × <i>ecalcarata</i> (three plants)	67	Meiosis appeared regular in all stages of division
<i>ecalcarata</i> × <i>shockleyi</i>	—	Seeds were produced but failed to germinate
<i>shockleyi</i> × <i>ecalcarata</i> (six plants)	41 ± 4.7	Five metaphase I cells had univalents and two anaphase I cells showed unequal segregation of chromosomes (6 + 8)
<i>sibirica</i> × <i>ecalcarata</i>	64 ± 6.4	Meiosis appeared regular in all stages of division
<i>ecalcarata</i> × <i>skinneri</i>	—	Fertilization occurred but seeds aborted prior to maturity
<i>skinneri</i> × <i>ecalcarata</i>	—	Seedlings were weak and died

TABLE III. Continued.

Hybrid combinations	Mean percent and standard error of stainable pollen	Observations of meiotic behavior or point of hybrid failure
<i>ecalcarata</i> × <i>viridiflora</i>	63 ± 2.9	Meiosis appeared regular in all stages of division
<i>viridiflora</i> × <i>ecalcarata</i>	61 ± 3.6	Meiosis appeared regular in all stages of division
<i>vulgaris</i> × <i>ecalcarata</i>	79 ± 4.6	Meiosis appeared regular in all stages of division
<i>flabellata</i> × <i>flavescens</i>	61 ± 2.8	Meiosis appeared regular in all stages of division
<i>flabellata</i> × <i>viridiflora</i>	45 ± 4.4	Meiosis appeared regular in all stages of division
<i>viridiflora</i> × <i>flabellata</i>	58 ± 3.9	Meiosis appeared regular in all stages of division
<i>flabellata</i> var. <i>alba</i> × <i>flavescens</i>	17 ± 2.5	Meiosis appeared regular in all stages of division
<i>flavescens</i> × <i>flabellata</i> var. <i>alba</i>	65 ± 5.7	Meiosis appeared regular in all stages of division
<i>flabellata</i> var. <i>alba</i> × <i>oxysepala</i> var. <i>yabeana</i>	(hybrids were pollen sterile)	Meiotic analyses could not be completed
<i>flabellata</i> var. <i>alba</i> × <i>viridiflora</i>	65 ± 4.4	Meiosis appeared regular in all stages of division
<i>viridiflora</i> × <i>flabellata</i> var. <i>alba</i>	65 ± 4.1	Meiosis appeared regular in all stages of division
<i>flavescens</i> × <i>olympica</i>	—	Fertilization occurred but seeds aborted prior to maturity
<i>flavescens</i> × <i>oxysepala</i> var. <i>yabeana</i>	—	Seeds were produced but failed to germinate*
<i>oxysepala</i> var. <i>yabeana</i> × <i>flavescens</i>	—	Fertilization occurred but seeds aborted prior to maturity†
<i>sibirica</i> × <i>flavescens</i>	57 ± 4.8	Meiosis appeared regular in all stages of division
<i>flavescens</i> × <i>viridiflora</i>	—	No apparent fertilization
<i>viridiflora</i> × <i>flavescens</i>	42 ± 5.5	Two metaphase I cells had a pair of univalents; anaphase I cells showed equal segregation of chromosomes
<i>longissima</i> × <i>olympica</i>	39 ± 3.7 (occasional giant pollen grains were observed)	Meiosis appeared regular in all stages of division

† The pollen tube squash technique was used to confirm pollen tube growth in the style of the seed parent.

TABLE III. Continued.

Hybrid combinations	Mean percent and standard error of stainable pollen	Observations of meiotic behavior or point of hybrid failure
<i>olympica</i> × <i>longissima</i>	40 ± 6.3 (occasional giant pollen grains were observed)	Meiosis appeared regular in all stages of division
<i>longissima</i> × <i>oxysepala</i> var. <i>yabeana</i>	—	Seeds were produced but failed to germinate*
<i>oxysepala</i> var. <i>yabeana</i> × <i>longissima</i>	—	Fertilization occurred but seeds aborted prior to maturity
<i>longissima</i> × <i>shockleyi</i>	91 ± 3.5	Meiosis appeared regular in all stages of division
<i>longissima</i> × <i>sibirica</i>	61 ± 4.1 (two giant pollen grains were observed)	Meiosis appeared regular in all stages of division
<i>longissima</i> × <i>skinneri</i>	64 ± 5.8	Meiosis appeared regular in all stages of division
<i>longissima</i> × <i>viridiflora</i>	13 ± 2.7 (pollen grains were highly variable in size, some 2n or larger)	Metaphase I cells frequently contained univalents, sometimes as many as 4, some of which appeared to be heteromorphic
<i>viridiflora</i> × <i>longissima</i>	—	Hybrids were weak and died prior to maturity
<i>longissima</i> × <i>vulgaris</i>	55 ± 5.7 (several giant pollen grains were observed)	Meiotic material was poor but apparently regular
<i>vulgaris</i> × <i>longissima</i>	—	No apparent fertilization
<i>oxysepala</i> var. <i>yabeana</i> × <i>olympica</i>	—	Seeds were produced but did not germinate
<i>shockleyi</i> × <i>olympica</i>	57 ± 7.1 (two giant pollen grains were observed)	Meiosis appeared regular in all stages of division
<i>skinneri</i> × <i>olympica</i>	59 ± 5.9 (two giant pollen grains were observed)	Meiosis appeared regular in all stages of division
<i>olympica</i> × <i>viridiflora</i>	54 ± 4.5 (occasional giant pollen grains were observed)	Meiosis appeared regular in all stages of division
<i>viridiflora</i> × <i>olympica</i>	58 ± 5.0	No analyses of meiosis were made
<i>oxysepala</i> var. <i>yabeana</i> × <i>shockleyi</i>	—	Fertilization occurred but seeds aborted prior to maturity
<i>shockleyi</i> × <i>oxysepala</i> var. <i>yabeana</i>	—	Seeds failed to germinate*
<i>sibirica</i> × <i>oxysepala</i> var. <i>yabeana</i>	84 ± 3.5	Meiosis appeared regular in all stages of division

TABLE III. Continued.

Hybrid combinations	Mean percent and standard error of stainable pollen	Observations of meiotic behavior or point of hybrid failure
<i>oxysepala</i> var. <i>yabeana</i> × <i>skinneri</i>	—	Fertilization occurred but seeds aborted prior to maturity
<i>skinneri</i> × <i>oxysepala</i> var. <i>yabeana</i>	—	Seeds were produced but failed to germinate*
<i>oxysepala</i> var. <i>yabeana</i> × <i>viridiflora</i>	—	Fertilization occurred but seeds aborted prior to maturity†
<i>viridiflora</i> × <i>oxysepala</i> var. <i>yabeana</i>	57 ± 4.4	Meiosis appeared regular except for the two occurrences of metaphase I univalents
<i>oxysepala</i> var. <i>yabeana</i> × <i>vulgaris</i>	—	No apparent fertilization
<i>vulgaris</i> × <i>oxysepala</i> var. <i>yabeana</i>	74 ± 3.9	Meiosis appeared regular in all stages of division
<i>shockleyi</i> × <i>skinneri</i>	93 ± 2.7	Meiosis appeared regular in all stages of division
<i>viridiflora</i> × <i>shockleyi</i>	65 ± 4.3 (several giant pollen grains were observed)	Meiosis appeared regular in all stages of division
<i>shockleyi</i> × <i>vulgaris</i>	67 ± 4.6	No analyses of meiosis were made
<i>vulgaris</i> × <i>shockleyi</i>	60 ± 5.2	Meiosis appeared regular in all stages of division
<i>skinneri</i> × <i>viridiflora</i>	62 ± 4.6 (occasional giant pollen grains were observed)	Meiosis appeared regular at all stages of division
<i>viridiflora</i> × <i>sibirica</i>	61 ± 6.2 (occasional giant pollen grains were observed)	Meiosis appeared regular at all stages of division
<i>skinneri</i> × <i>viridiflora</i>	—	Seeds were produced but failed to germinate
<i>viridiflora</i> × <i>skinneri</i>	—	Seeds were produced but failed to germinate
<i>vulgaris</i> × <i>skinneri</i>	62 ± 3.0	Metaphase I cells were not observed but anaphase I cells showed numerically equivalent segregation of chromosomes
<i>viridiflora</i> × <i>vulgaris</i>	61 ± 9.8 (several giant pollen grains were observed)	Meiosis appeared regular at all stages of division
<i>vulgaris</i> × <i>viridiflora</i>	55 ± 9.9 (numerous giant pollen grains were observed)	No analyses of meiotic behavior were made

The following is a discussion of inheritance patterns of F_1 hybrids regarding the more important characteristics considered in this study.

Height: With few exceptions, the hybrids were as tall as, or taller than, the more robust parental plant. From this observation it would appear that tallness is dominant over compact habit. However, the situation is complicated in many hybrid combinations by the occurrence of hybrid vigor or heterosis. In the few exceptions noted in which the hybrids were inferior to both parents in height, they were weak in other respects and never reached maturity.

Branching habit: The degree of branching is, in general, intermediate between that of parental species which differ significantly in this characteristic. However, when crosses were made between two much-branched, many-flowered forms, the resulting offspring often exceeded either parent in the extent of branching.

Leaves: The biternate basal leaf appears to be dominant over both the ternate and triternate. It should be noted, however, that these three characteristics form a quantitative series with the biternate leaf being intermediate between the other two.

Blue-green leaves are characteristic of a few species, e.g., *A. flabellata* and *A. sibirica*. This is a dominant trait and can be observed in all F_1 hybrids involving one of these species.

In *Aquilegia* petiolate cauline leaves are never numerous and seldom lacking. The cauline leaves of the hybrids are in general intermediate in number between those of the parents.

Floral posture: Nodding flowers are dominant over erect ones. If either parental species had nodding flowers, the hybrids without exception were similarly characterized. There is, however, some variation in expression. For example, flowers of hybrids involving *A. longissima* are typically semi-erect or less strongly nodding than would be expected if dominance were complete. The expression of the genetic factor, or factors, involved in floral posture is therefore thought to be altered by the action of modifiers or suppressors.

Petal morphology: Hooked spurs are dominant over straight spurs. However, the degree of curvature, as well as spur length and width, shows continuous variation. The spurless condition in *A. ecalcarata* is recessive, and spurs were present in all hybrids involving this species.

Genetic factors controlling length and spreading tendency of the laminae are likely pleiotropic and the dominant condition seems to be long, slightly spreading laminae.

Floral color: The color patterns of the sepals and spurs strongly indicate that genic dominance is incomplete or lacking and in most cases pigmentation of both parents is represented in the hybrids with the darker colors partially or completely masking the lighter.³ However, there does appear to be some dominance of lighter colors, especially yellow, over dark colors in laminae, although it is certainly not complete.

*Hybrid Behavior*⁴

Normally 12 hybrid plants of each cross were grown to maturity. All hybrids sampled had a diploid chromosome number of 14. Most interspecific crosses resulted

³ Schafer (1941) and Kristofferson (1920) both reported simple Mendelian segregation of dominant phenotypes concerning inheritance of flower color of *A. vulgaris*. Also, the data of Prazmo (1965a), which were derived from an extensive hybridization study in which F_2 segregation ratios were determined, indicate that there exists an interaction between four pairs of alleles each of which is inherited independently. Prazmo does, however, conclude that there are other factors "modifiers or inhibitors" which alter the expression of these four genes.

⁴ Complete hybrid descriptions have been recorded and may be obtained from me.

in the production of viable and relatively fertile F_1 plants. However, hybrids of some species widely separated geographically and very dissimilar morphologically proved to be quite infertile, and in extreme cases hybrids were not produced or did not reach maturity. All hybrid plants grown to maturity produced seeds, the number of which varied according to the hybrid combination.

Table III is a summary presentation of pollen stainability, meiotic behavior, and indicates the point at which hybridization between certain species failed. To facilitate comparison of reciprocal results, the hybrid combinations are arranged alphabetically according to seed parent, with the reciprocal, if attempted, immediately following the first entry.

DISCUSSION

Because of its cytological uniformity and lack of natural polyploidy,⁵ *Aquilegia* has often been considered a genus at a youthful stage of evolution (Clausen, Keck, & Hiesey, 1945; Stebbins, 1950, 1965; Grant, 1952; and others). It has been assumed by several authors (Payson, 1918; Winge, 1928; Anderson, 1931; Grant, 1952; Pelton, 1957; and others) that all species of *Aquilegia* can be hybridized with the production of fertile progeny. The data derived from the present study and that of Prazmo (1965a, 1965b) indicate that partial or complete genetic barriers do exist and that crossability, as would be expected, is closely correlated with geographic distribution.

Today the least specialized and presumably more primitive species are found in moderately cool habitats. It is very difficult to determine, however, which of the extant species represents the oldest taxon. A good possibility is that *A. vulgaris* with its numerous varieties represents an ancestral group from which most, or all, other existing species are derived either directly or indirectly. Schafer (1941) points out that all of the genetic factors affecting several color variations which she was able to observe in six species of *Aquilegia* were to be found in *A. vulgaris*. She felt that this fact was creditable evidence for the ancestral position of *A. vulgaris*. In another paper (Anderson & Schafer, 1931) it was stated that regardless of which of several species were hybridized, the offspring resembled *A. vulgaris* in color pattern and general morphology. This observation supposedly added support to an ancestral position for *A. vulgaris*. Observations made during the present study indicate that most key characteristics exhibit continuous variation and that the expression of these same characteristics are intermediate in *A. vulgaris*. Also, the plants of this complex have nodding flowers and hooked spurs, both of which are clearly dominant, and most have dark floral colors which tend to mask the expression of lighter hues. Therefore, plants of various hybrid combinations would superficially resemble *A. vulgaris*. The important point, however, is not that hybrids resemble *A. vulgaris*, but that *A. vulgaris* resembles intermediate hybrids. The intermediate nature of *A. vulgaris* implies nonspecialization which in turn is suggestive of an ancestral status.

Possibly the best evidence in support of an ancestral status for *A. vulgaris* is its genotypic intermediacy, i.e., it appears to be a link between genetically isolated or nearly isolated species. In the present study, with one exception,⁶ all attempts to cross *A. vulgaris* with other species were successful, and in no case was the pollen fertility of the hybrids less than 50%. Genetically, *Aquilegia* appears to be a good example of a Rassenkreis or system of polytypic species with *A. vulgaris* occupying a central position.

⁵ An allotetraploid species was produced by Skalinska (1935) in a controlled hybridization program.

⁶ A single cross involving *A. vulgaris* failed to result in the production of mature hybrid plants, that of *A. vulgaris* × *A. longissima*. In this case fertilization probably did not occur.

An alternative and very popular view is that *Aquilegia ecalcarata* is the most primitive living representative of the genus (Drummond & Hutchinson, 1920; Skalinska, 1958; Prazmo, 1965a, 1965b; Stebbins, 1965). Arguments in favor of this idea are based primarily on the morphological similarity of the flowers of *A. ecalcarata* to those of *Isopyrum* and the absence of spurs (the spurs of *A. ecalcarata* are represented by small pouch-like glands which are similar to nectary glands found at the base of the petals of some primitive genera of Ranunculaceae).

Experiments conducted by Skalinska (1958) confirm a distant relationship between these two taxa but her data contribute nothing toward the elucidation of interspecific relationships in *Aquilegia*, i.e., *Isopyrum* may be more closely related to other species of *Aquilegia*.

Obviously the ancestors of *Aquilegia* must have been spurless. This condition is not, however, restricted to *A. ecalcarata*; there are spurless representatives in the Vulgaris Complex. As a point of fact, there is a continuum of representation from the spurless to the normal spurred condition. Spurlessness as it occurs in *A. ecalcarata* is expressed as a function of one or two pairs of recessive alleles (Prazmo, 1965a), and all *A. ecalcarata* hybrids obtained in the present experiments have been spurred. Although spurs could have originated through modification of the glands (nectaries) at the base of the petals following a dominant mutation, it seems more likely that a part as diverse in structure and function as the Columbine spur would have evolved slowly in response to selective pressures over a long period of time. Spurlessness in *A. ecalcarata* then could result from a recessive loss of some essential step in the normal sequence of spur development.

On the basis of crossability and pollen fertility determinations of this study, *A. ecalcarata* is most closely related to *A. vulgaris*, *A. atrata*, *A. alpina*, and *A. oxysepala* var. *yabeana*, and least closely related to North American Columbines. This is generally in accord with the data of Prazmo (1965b).

On morphologic and genetic evidence, the central position of *A. vulgaris* seems clear, whereas the position of *A. ecalcarata* seems peripheral. Whether *A. vulgaris* indeed is ancestral or has arisen secondarily through hybridization is a question on which there is no conclusive evidence at present. If it is not ancestral, there is no existing species which so nearly meets the requirements of a primitive *Aquilegia*. *A. ecalcarata* seems to be a conspicuously poor candidate in spite of its spurless petals.

Assuming that *A. vulgaris* is ancestral, initial evolution toward the development of the Eastern Asiatic and North American Columbines probably followed a common pathway. Later in the phylogeny of the genus, this pathway forked at or about the time *A. alpina* originated. This taxon hybridizes readily with *A. vulgaris*, *A. sibirica* (one of the more primitive of the Asiatic species), and with various American species. The hybrids produced from these crosses are characterized by having a relatively high pollen fertility.

Further branching of the Asiatic line resulted in the formation of a group of primarily blue-flowered Columbines and various purple-flowered forms. The blue-flowered group is represented in the present study by *A. sibirica* and *A. flabellata*, the latter probably being a direct derivative from the former. Two purple-flowered forms which represent terminal taxa of different branches are *A. oxysepala* var. *yabeana* and *A. ecalcarata*. Data from the present study show *A. oxysepala* var. *yabeana* to be isolated genetically from all American species. With all other species it was successfully crossed as the pollen parent. The only species with which it could be hybridized as the seed parent, however, was *A. ecalcarata*.

Aquilegia viridiflora, an Asiatic species of uncertain derivation, is unusual not only because of its morphology but also because of the inconsistency of its ability to hybridize with species which appear closely related. For example, it hybridizes

readily with some species of North American Columbines, with fertile hybrids being produced. With other American species, it does not cross or the hybrids are weak and do not develop normally.

In previous treatments, *A. viridiflora* has been placed in the Alpina group, primarily because of its straight spurs. Its apparent lack of crossability with *A. alpina*, however, does not support a relationship between the two taxa. On the basis of crossability and hybrid fertility, *A. viridiflora* appears to be most closely related to *A. atrata* and *A. vulgaris* and least closely related to members of Canadensis and Caerulea Complexes.

Aquilegia brevistyla is a member of a complex which, according to Munz, is more closely allied to Eurasian species than to other American species. *Aquilegia brevistyla* itself shares the following characteristics with *A. sibirica* and *A. flabellata*: short spurs, included stamens, blue and white floral color, short styles, and structurally similar follicles. Also, the leaves are similar in having broad leaflets with slight blue-green coloration. It is a very attractive hypothesis that *A. brevistyla* represents a link between Old World and New World Columbines. On the basis of crossability and pollen fertility of the hybrids, however, the affinities of *A. brevistyla* lie within the American complexes.

The Caerulea Complex is represented by *A. longissima* and *A. caerulea*. On the basis of its interspecific crossability and the high pollen fertility of its hybrids, *A. caerulea* appears to be one of the more primitive of American Columbines. It is also very closely related to *A. brevistyla*. *Aquilegia longissima* is more specialized although obviously closely related to *A. caerulea*.

The other American complex consists of species which probably represent the primary evolutionary pathway of American species or are terminal taxa on short branches from the major pathway. These species are *A. canadensis*, *A. formosa*, and their near allies.

The uninhibited crossability of species within and among American complexes, coupled with high pollen fertility of all F₁ hybrids, indicates that the American species are all very closely related with less genotypic specialization than has occurred in Old World species.⁷ It follows that the American species constitute a younger and more recently evolved group of Columbines. Based upon the data derived from the present study, a chart of the phylogeny of *Aquilegia* has been proposed (Fig. 1).

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⁷Prazmo (1965b) presented a phylogenetic scheme of five complexes: *Vulgaris*, *Ecalcarata*, *Alpina*, *Canadensis*, and *Caerulea*. The latter two are shown to be the most closely related, the *Ecalcarata* and *Caerulea* Complexes the most distantly related.

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