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ISOLATION AND HYBRIDIZATION BETWEEN *AQUILEGIA FORMOSA* AND *A. PUBESCENS*

VERNE GRANT*

The columbines have long been noted as a genus with little or no sterility or incompatibility between dissimilar morphological types. Fertile and vigorous hybrids have been obtained by geneticists and plant breeders from nearly every cross that has been attempted in the genus. Spontaneous garden hybrids are of frequent occurrence in the cultivated columbines. In nature, also, hybridization apparently occurs with some frequency. The purpose of the present paper is to describe a case of natural hybridization between two species of columbine, *Aquilegia formosa* and *A. pubescens*, in the Sierra Nevada of California, and, in the light of this example, to discuss the species problem in the genus *Aquilegia* as a whole.

The problem was suggested to the author by Dr. Jens Clausen of the Carnegie Institution of Washington. The field work was made possible by a visit to the Timberline transplant station of that institution during the summer of 1950. Dr. Clausen guided the author to the hybrid colony and subsequently furnished many helpful suggestions concerning the work. The author has similarly benefitted from conversations with Dr. Philip A. Munz of the Rancho Santa Ana Botanic Garden, who generously placed many unpublished distribution records of *Aquilegia* at his disposal. The manuscript was read and criticized by these two authorities and by Dr. Edgar Anderson of the Missouri Botanical Garden. The author wishes to acknowledge his gratitude to these men for their interest and helpful criticisms.

THE HYBRID COLONY

Aquilegia formosa Fisch., the common red Columbine of western North America, is represented throughout the Sierra Nevada of California by two varieties known as *truncata* and *pauciflora*, which occur mostly at elevations below 10,000 feet. In a relatively small area in the southern Sierra Nevada, at elevations above 9000 feet, there occurs a second kind of columbine, the yellow-flowered, long-spurred *Aquilegia pubescens* Coville. The ranges of the two entities overlap at various points and there hybridization occurs.

One such region of overlap, and the one which was explored in detail in connection with the present study, lies in the Harvey Monroe Hall Natural Area near Tioga Pass on the eastern boundary of Yosemite National Park. This area of about 9 square miles, which includes the Timberline station maintained by the Carnegie Institution of Washington, has been described by Clausen, Keck and Hiesey (1940, chap. i). Slate Creek Valley runs through

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the center of the area at an elevation of about 10,000 feet. It is surrounded by barren peaks which attain heights of 11,000 to 12,500 feet. One fork of Slate Creek drains Saddlebag Lake, a glacial lake on the east side of the area.

Aquilegia formosa is common on sunny hillsides and ravines of Slate Creek Valley at elevations around 10,000 feet. *Aquilegia pubescens* occurs in cold places, often under overhanging rocks, on the northern and eastern slopes of the surrounding peaks and ridges. It is usually found at elevations between 10,500 and 11,500 feet in this region. On the northeast-facing slope of a ridge adjoining Saddlebag Lake, plants of *A. pubescens* have descended to about 10,200 feet, where they have met with plants of *A. formosa* ascending from nearby ravines in Slate Creek Valley. This is the site of the hybrid colony to be described in the following paragraphs.

The colony, which was first reported by Clausen, Keck and Hiesey (1945, p. 78), numbers about 65 individuals and occupies an area about 75 feet long and 50 feet wide on the slope by Saddlebag Lake. Several individuals of *A. formosa* are congregated at the lower end of the colony, some individuals of *A. pubescens* occur at the upper end, and a varied lot of intermediate types is strung out between them. All of the individuals in the colony, intermediates as well as parental types, set an abundance of sound seeds.

A mature flower on each one of 57 individual plants in this colony was measured for five different characters. The flowers were later preserved as a mass collection. Similar measurements were made on samples of *A. formosa* in Slate Creek Valley and *A. pubescens* on the higher slopes of White Mt. In order to analyze the composition of both the hybrid colony and the species populations in the same area, it was necessary to know the characteristics of the two entities previous to hybridization. An estimate of the characters of pure *formosa* was obtained from measurements of herbarium specimens of this entity collected in the northern part of the Sierra Nevada away from any present direct contact with *A. pubescens*. A comparable method could not be employed to learn the characters of pure *pubescens*, due to the fact that no part of the range of this entity lies outside the distribution area of *A. formosa* and hence away from possible contamination by hybridization. The characters of pure *pubescens* therefore had to be inferred somewhat indirectly from measurements of the most extreme individuals in the White Mt. and Saddlebag Lake populations. The procedure used for defining the limits of pure *pubescens* will be explained in more detail in a subsequent paragraph.

The floral characters differentiating pure *formosa* and pure *pubescens* are summarized in table 1 and illustrated in figure 1. *Aquilegia formosa* in the northern Sierra Nevada has nodding flowers with bright red spurs and sepals and deep yellow petal blades; the spur is short and straight (10 to 17 mm. long); and the petal blade is likewise short (2 to 4 mm.). Pure *pubescens* is considered to have erect flowers with white or pale yellow spurs and petal blades; long, slender, gently curving spurs (29 to 37 mm.); and long petal blades (9 to 12 mm.).

Index values were assigned to these characters in such a way that pure *formosa* received a total score of 0, pure *pubescens* a score of 10, and intermediate types various scores from 1 to 9 (see Anderson, 1949). Five characters

were used in this comparison, as shown in table 1; a sixth character, curvature of the spur, proved difficult to score objectively and was not used. The score value, or so-called hybrid index, was computed for each individual in

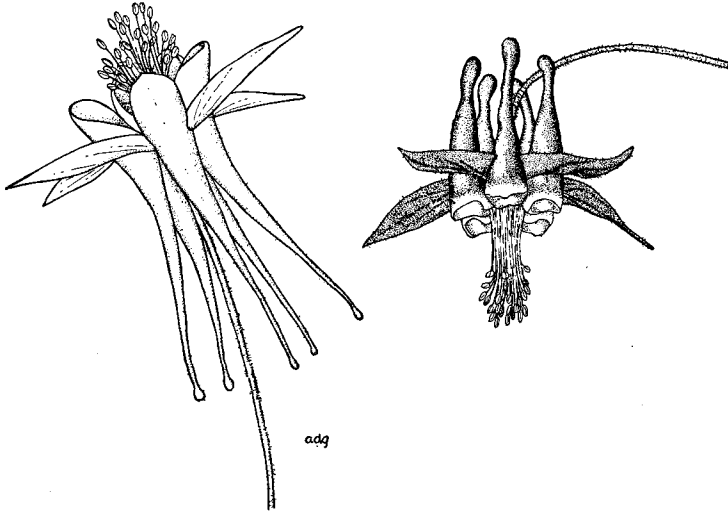


Figure 1. The flowers of *Aquilegia pubescens* (left) and *A. formosa* var. *truncata* (right).

the colony at Saddlebag Lake, and the percentage distribution of these hybrid indices was plotted in the form of a bar graph (fig. 2.).

Reference to this figure shows that about 5 per cent of the individuals in the Saddlebag Lake population are morphologically like pure *formosa* in the northern Sierra Nevada, and that about 3.5 per cent of the individuals

TABLE 1. COMPARISON OF *AQUILEGIA FORMOSA* AND *A. PUBESCENS* WITH RESPECT TO FIVE FLORAL CHARACTERS AND THEIR INDEX VALUES

Character	<i>A. formosa</i> (n. Sierra Nevada)	Intermediates	<i>A. pubescens</i> (extreme type)
Flower position	nodding	horizontal	erect
Spur and sepal color	red	orange, pale orange, pink, or pale pink	pale yellow, or white
Petal blade color	deep yellow	medium yellow	pale yellow, or white
Spur length	10-17 mm.	18-28 mm.	29-37 mm.
Petal blade length	2-4 mm.	5-8 mm.	9-12 mm.
Index value for each character	0	1	2
Total index value	0	5	10

have the characters of *pubescens* in its extreme condition. The remaining individuals in the colony represent various intermediate conditions having index values between 1 and 9.

The frequencies of the intermediate types in the natural colony do not form a random distribution as in an experimental F₂ family, but rather appear to be grouped into several fairly discrete contingents. There is the "pure"

formosa contingent which has already been mentioned. The individuals of extreme *pubescens* are grouped with a much larger number of *pubescens*-like individuals into a second contingent, which constitutes 75 per cent of the population. A third contingent, comprising 17 per cent of the plants in the colony, consists of individuals which are plainly hybrid intermediates. Finally, there is a small percentage of recombination types. The Saddlebag Lake colony is composed, in short, of a few individuals of *A. formosa*, a large number of individuals of *A. pubescens*, a relatively small number of hybrid intermediates, and a few recombination types.

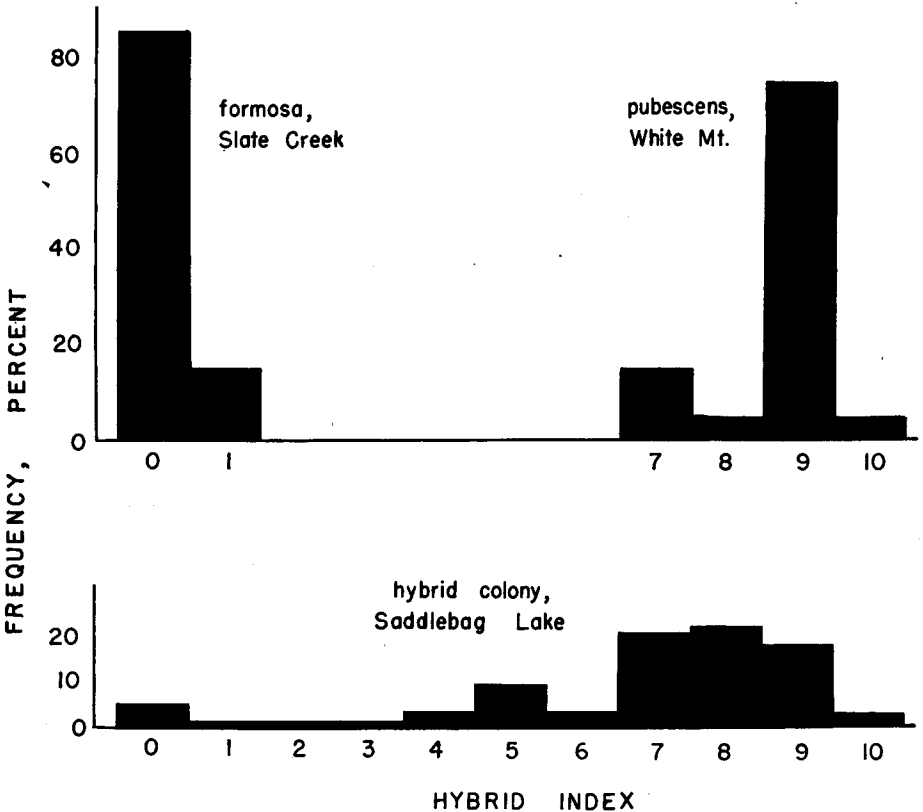


Figure 2. Bar graphs showing the frequency distribution of different hybrid index values, computed from table 1, in three populations of *Aquilegia* in the high Sierra Nevada.

The computation of the hybrid index, involving as it did a grouping of varied individuals into ten broad classes, resulted in a considerable loss of the original data. It seemed worthwhile, therefore, to supplement the bar graph (fig. 2) with a polygonal graph in which the quantitative aspect of the data could be preserved. Such a series of polygonal graphs, in which the range of variation in three floral characters is shown for several populations, is presented in figure 3.

The first polygonal graph (fig. 3A) shows the range of variation in pure

formosa compared with that in extreme *pubescens*. This graph may be compared with fig. 3C, which shows the overlapping variation patterns of the parental and hybrid contingents in the Saddlebag Lake colony. Not all of the hybrids at Saddlebag Lake were intermediates; there were a few recombination types, and these have been plotted separately in figure 3D. It will be evident from an examination of the latter graph that no radical recombinations between the characters of *A. formosa* and *A. pubescens* have occurred. This fact is brought out also in table 2, where the characters of the plants in the hybrid colony are tabulated. The most extreme recombination types to be found in the colony are plants which conform to a parental type in one or two characters and are intermediate in the other characters. No plant in the Saddlebag Lake colony has combined a character of *A. formosa* with a character of *A. pubescens*.

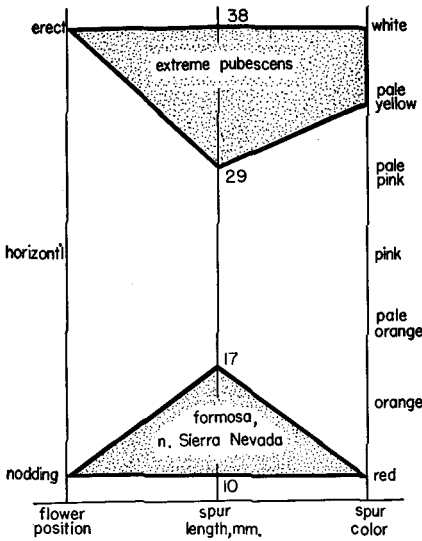
In order to obtain further information about recombination between the parental characters in the hybrid colony, the five most variable floral characters, those listed in table 1, were paired in different combinations and studied for correlation. The ten correlation coefficients obtained from these calculations range in magnitude from 0.62 to 0.81 and average 0.71. With a sample of 57 individuals, these values of r are highly significant and cannot be explained on the basis of chance alone. This means that the five characters of the two entities have a strong tendency to stick together. Observations of a sixth character which could not be readily measured, curvature of the spur, suggest that it is probably strongly correlated with the other floral characters. These facts indicate that the characters of the two entities are not breaking up as a result of hybridization.

INTROGRESSION

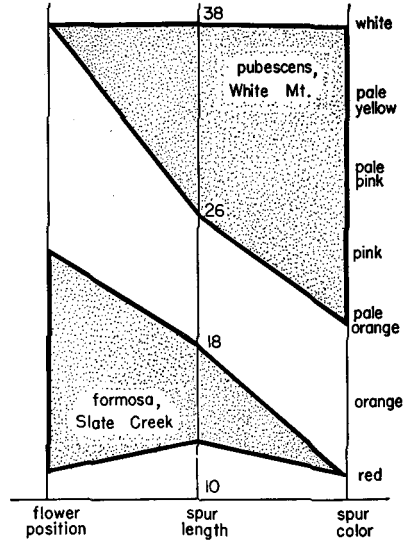
In the preceding section grounds were given for believing that hybridization between *A. formosa* and *A. pubescens* is not resulting in a general dissolution of the discontinuities between those two entities. The evidence to be presented next purports to show that the main result of this hybridization is the introgression or infiltration of genes from one entity into the other by means of repeated backcrossing (see Anderson, 1949).

It has already been noted that the *pubescens* contingent in the hybrid colony consists of a few individuals of extreme *pubescens* together with a much larger number of *pubescens*-like individuals with index values of 7, 8 and 9. The former plants have yellow or white flowers and long spurs (29 to 38 mm.), while the latter have pink or pale pink flowers and shorter spurs (down to 25 mm.). On the basis of their morphological characters, the pink-flowered plants with spurs of medium length are judged to represent various backcrosses to extreme *pubescens*. That is to say, they are types of *A. pubescens* which have acquired some genes from *A. formosa*.

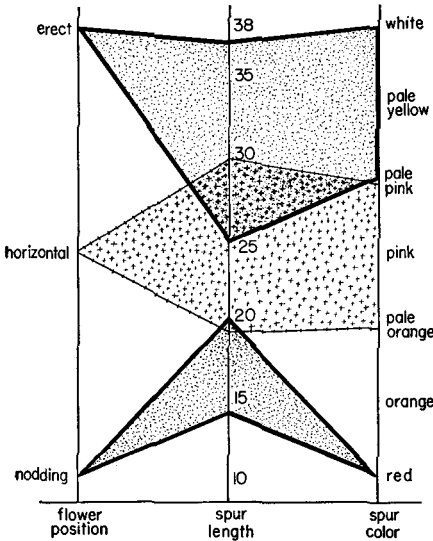
In the light of this hypothesis, it is significant that plants with the same characteristics of pink-colored flowers and medium-long spurs can be found in the population of *A. pubescens* on White Mt., which is not in direct contact with *A. formosa*. Here, as at Saddlebag Lake, the extreme *pubescens* types are in a minority (see fig. 2). Unlike Saddlebag Lake, however, the types with pink flowers and medium-long spurs are also in a minority in the White



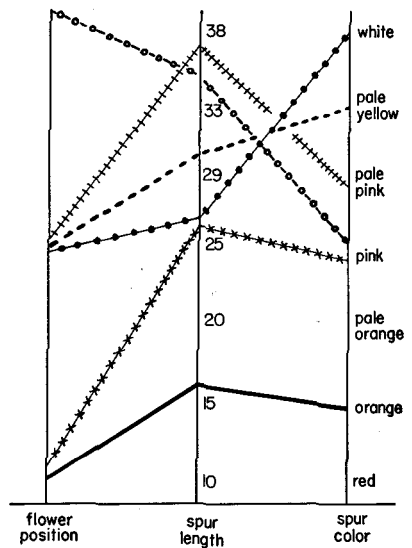
(A) Extreme types of the species



(B) Introgressive types of the species



(C) Parental & intermediate types in the hybrid colony at Saddlebag Lake



(D) Six recombination types in the colony at Saddlebag Lake

Figure 3. Polygonal graphs showing the range of variation for three floral characters in several populations of *Aquilegia* in the Sierra Nevada.

Mt. population, and most of the individuals have pale pink flowers with long spurs. The frequent occurrence of pale pink flowers in the population on White Mt., as well as the occasional occurrence of other variations in the direction of *A. formosa* (see fig. 3B), suggests that the characters of this population have been affected by introgression. On the other hand, the great frequency of individuals with the high index value of 9, as compared with the greater variance and lower average index values in the Saddlebag Lake population (see fig. 2), suggests that the effects of introgression have here been greatly restricted by counteracting forces such as environmental selection.

It appears from a study of herbarium specimens that there is no part of the range of *A. pubescens* where yellow or white-flowered plants form a pure stand. Individuals with pale pink flowers occur intermixed with yellow-flowered individuals throughout the southern Sierra Nevada. Since hybridization between *A. pubescens* and *A. formosa* is not confined to the single locality at Saddlebag Lake, but occurs elsewhere in the Sierra Nevada, as will be brought out later (p. 356), this result might be expected, and can be simply explained by saying that the germ plasm of *A. pubescens* has been contaminated by introgression from *A. formosa* in every part of its range.

Aquilegia pubescens has, however, some close relatives in the desert ranges of the southwest which are geographically isolated from any red-flowered columbine. Populations of *A. chrysantha*, *A. Chaplinei*, *A. Hinckleyana*, and *A. longissima* are apparently quite uniform in the possession of yellow flowers with long spurs (30 mm. or more). The decision that pure *pubescens* is a plant with yellow flowers and long spurs is based partly upon analogy with the conditions in its closest systematic relations in areas remote from possible introgression. In setting the lower limit of spur length at 29 mm. for computing the hybrid index, the author was also partly influenced by the observation that most of the individuals in the White Mt. and Saddlebag Lake populations with erect and yellow-colored flowers also had spurs at least this long.

The existence of about seven fairly discrete phenotypic classes for spur color in the hybrid colony at Saddlebag Lake suggests that the two parental entities may differ in about three genes governing this character. The action of the genes within either parental entity might well be to turn some precursor substance in the flower buds into either a red anthocyanin pigment or a yellow anthoxanthin. The mixture of gene-controlled reactions for both anthocyanin and anthoxanthin production in the hybrids would then lead to a competition for the limited quantities of their common precursor, and, depending upon the relative rates of the different reactions, would result in phenotypes with some intermediate value (see Lawrence and Price, 1940). The fact that the pale colors of *A. chrysantha* tend to suppress the bright colors of red columbines in hybrid combinations (Anderson and Schafer, unpubl.) may mean that anthoxanthin production proceeds at a faster rate than anthocyanin formation. Whether the same dominance relations hold between Sierran strains of *A. pubescens* and *A. formosa* is not known. The subdued pink hues which are so common in populations of *A. pubescens* could easily be explained, however, on the basis of a suppression of anthocyanin formation by anthoxanthins in individuals heterozygous for the respective genes.

There is some evidence that genes suppressing anthocyanin formation have infiltrated into the Slate Creek population of *A. formosa*. When plants from this population were compared with plants of *A. formosa* in Leevining Canyon, which is at the lower altitude of 8500 feet and a couple of miles outside the range of *A. pubescens*, it was found that the red of the Slate Creek flowers, which are surrounded by *A. pubescens*, was a shade less bright than that of the Leevining Canyon flowers. This observation, together with the range and pattern of variation for some other characters in the Slate Creek population (see figs. 2 and 3B), suggests that genes of *A. pubescens* have migrated into some local populations of *A. formosa* in the high Sierra Nevada, and that introgression has therefore been a reciprocal process in this region.

TABLE 2. TABULATION OF CHARACTER COMBINATIONS IN THE HYBRID COLONY AT SADDLEBAG LAKE

Flower position	Spur and sepal color	Combination of characters			No. of individuals
		Petal blade color	Spur length, mm.	Petal blade length, mm.	
nodding	red	deep yellow	10-17	2-4	3
	red	deep yellow	18-28	5-8	1
	orange	deep yellow	10-17	2-4	1
	pink	medium yellow	18-28	5-8	1
	pink	pale yellow	18-28	5-8	1
horizontal	pale orange	deep yellow	18-28	2-4	1
	pale orange	medium yellow	18-28	2-4	1
	pale orange	medium yellow	18-28	5-8	1
	pink	medium yellow	18-28	5-8	3
	pink	pale yellow	18-28	5-8	2
	pink	pale yellow	18-28	9-12	1
	pink	pale yellow	29-37	9-12	1
	pale pink	pale yellow	18-28	9-12	4
	pale pink	pale yellow	29-37	9-12	1
	pale yellow	pale yellow	18-28	5-8	1
	pale yellow	pale yellow	29-37	9-12	1
	erect	pale orange	medium yellow	29-37	9-12
pale orange		pale yellow	18-28	5-8	1
pink		pale yellow	18-28	5-8	3
pink		pale yellow	29-37	5-8	3
pink		pale yellow	29-37	9-12	2
pale pink		pale yellow	18-28	5-8	1
pale pink		pale yellow	18-28	9-12	2
pale pink		pale yellow	29-37	5-8	4
pale pink		pale yellow	29-37	9-12	12
white		pale yellow	18-28	9-12	2
white		pale yellow	29-37	9-12	2

Total No. possible combinations 567

Total No. actual combinations 27

Total No. individuals 57

The introgressive populations of *A. formosa* and *A. pubescens* show greater variance and less conformity to their respective pure parental types for flower color than they do for length of spur or flower position. Between the initial generation of variability in all floral characters in the hybrid colony and the establishment of the variations in the neighboring populations, there evidently intervenes a factor of restriction, which operates in a more severe

fashion on flower position or spur length than on flower color. That restrictive factor may be natural selection operating at the stage of flower pollination. Some floral characters, such as the position or dimensions of the nectar-containing spurs, are probably adjusted to the habits and requirements of the animal pollinators within fairly narrow limits. Much wider variation is probably tolerated in the color of the spurs and sepals, since pollinating birds or insects will not discriminate between different flowers on account of relatively slight differences in color (Bené, 1946; Grant, 1950). The pale pink shades in the flowers of many individuals of *A. pubescens* constitute a marker of introgression from *A. formosa*, therefore, because this color variation, unlike most alterations in the floral form, is a relatively neutral character from the standpoint of natural selection.

The existence of introgressive hybridization between *Aquilegia formosa* and *A. pubescens*, in conclusion, seems reasonably well established. An important problem for future studies is how far the effects of this hybridization have extended outside the area of contemporary contact between the hybridizing entities.

POLLINATION

Observations made at Echo Lake, El Dorado County, in the northern Sierra Nevada, at Slate Creek and Saddlebag Lake, and in the San Jacinto Mountains of southern California, indicate that *Aquilegia formosa* is normally pollinated by hummingbirds. The principal pollinator of the red Columbine in the Sierra Nevada is the Rufous Hummingbird, *Selasphorus rufus*. This bird feeds on the nodding red flowers by hovering beneath them and thrusting its bill vertically upward into the spurs. It may probe each of the five spurs of a flower in rapid succession, or only some of them, before flying off to another flower or to a perching place. Since the stamens and styles are well exerted beyond the center of the whorl of spurs, the bird cannot help brushing its forehead or chin against them in the process of sucking the nectar. It undoubtedly carries the pollen from flower to flower on its head feathers.

Merritt observed hummingbirds visiting the flowers of *Aquilegia formosa* in the San Bernardino Mountains of southern California in 1896. Numerous observers have reported visits of the Ruby-throated Hummingbird, *Archilochus colubris*, to the red-flowered Columbine of eastern North America, *Aquilegia canadensis* (Todd, 1880; Schneck, 1901; Graenicher, 1910; Pickens, 1931, Bené, 1946; James, 1948). It is probable that the whole group of red-flowered columbines, *A. formosa*, *A. canadensis* and their allies, which is entirely confined to North America, is pollinated by hummingbirds. A Mexican species in this group, *A. Skinneri*, with pale reddish and greenish flowers and long spurs (35 to 50 mm.), may not be a hummingbird flower (Payson, 1918).

Aquilegia pubescens on White Mt. was observed during six hours on two successive days in August 1950. During the hours of daylight no animals were observed to visit the flowers of this species. At twilight, however, a hawkmoth, *Deilephila lineata*, appeared in the population and hovered over the erect white flowers to suck nectar from the long slender spurs. A moth engaged in this operation was collected and observed to have the yellow columbine pollen on its head. The same species of sphingid moth was subsequently found pol-

linating *Aquilegia chrysantha* in the Santa Catalina Mountains in southern Arizona.

Several botanists have predicted that the relatives of *A. pubescens*, namely *A. chrysantha*, *A. caerulea* and *A. longissima*, would prove to be pollinated by moths (Gray, 1883; Trelease, 1883; Knuth, 1898; Payson, 1918). The finding that *A. pubescens* and *A. chrysantha* are hawkmoth flowers strengthens these suppositions and, at the same time, emphasizes the desirability of broader observational data.

At Saddlebag Lake, where *A. formosa* and *A. pubescens* grow together, the behavior of the pollinating agents is in general true to pattern. The hummingbird flies by day and confines its visits to *A. formosa* and the *formosa*-like backcross types. If it visits the flowers of *A. pubescens* at all, it is in order to bite off their spurs, a type of visit which does not result in cross-pollination. Some 13 plants in the Saddlebag Lake colony had flowers with the spurs bitten off; 12 of these plants were *pubescens*-like individuals with long or medium-long spurs, and one was a *formosa*-like plant.

The hawkmoth comes out at twilight at Saddlebag Lake and flies to the clearly visible, erect flowers of *A. pubescens* and the *pubescens*-like backcross types, but pays no attention to the nearly invisible flowers of *A. formosa*. (The judgment that *A. pubescens* is clearly visible at a distance of 15 feet in the evening light, while the red flowers of *A. formosa* are invisible at more than a few inches, is of course that of a human observer.) The hawkmoth, which normally uncoils its proboscis while hovering in a more or less upright position, would probably experience some difficulty in approaching the inverted flowers of *A. formosa*, even if it could see them.

Some intermediate types in the Saddlebag Lake colony with horizontal bright pink flowers and medium-long spurs are visited and pollinated at different times by both the hummingbirds and the hawkmoths. These are probably the only plants in the colony that are regularly pollinated by both kinds of animal.

The existence of hybrid intermediates in the Saddlebag Lake colony proves that the floral isolating mechanism which separates the parental entities must occasionally break down. If the original cross-pollination between a *formosa* flower and a *pubescens* flower was not carried out by either a hummingbird or a hawkmoth, it must have been performed by some other animal. A clue as to the possible identity of this animal is provided by the finding of a pollen-collecting bumblebee on the flowers of *A. formosa* in the Saddlebag Lake colony. Since bumblebees are known to fly occasionally from one species of flower to another (see Grant, 1950), it is reasonable to suppose that they have done so here. Inconstant flower visits of the type required to produce an interspecific hybrid would be especially frequent, moreover, if only a few individuals of each kind of plant were present, as must have been the case when the talus slope by Saddlebag Lake was first colonized by *A. pubescens* and *A. formosa*.

Once the F_1 hybrid was formed, as an incidental result of the pollen-gathering activities of the bumblebees (or some other bees or flies), it would have been backcrossed to the *formosa* parent by the hummingbirds and to the

pubescens parent by the hawkmoths. The normal pollinators in each species of columbine are the agents of introgressive hybridization in every stage subsequent to the initial crossing.

ISOLATION

The natural hybrids between *A. formosa* and *A. pubescens* are fully fertile. If hybrid sterility is used as the sole criterion of the boundaries of species, therefore, as is done by a number of biologists, it would follow that *A. formosa* and *A. pubescens*, together with all their relatives in North America, should be regarded as members of one species. But the existence of a gap between the variation pattern of *A. formosa* and that of *A. pubescens* in an area where they meet and hybridize (fig. 3B), indicates that these two entities do not in fact freely exchange genes, but comprise instead two different systems of interbreeding populations (see also Epling, 1945). Some barriers to gene exchange other than hybrid sterility or incompatibility must exist between *A. formosa* and *A. pubescens*.

The first such barrier separating *A. formosa* and *A. pubescens* is ecological isolation. *Aquilegia formosa* inhabits damp sunny slopes at lower elevations and *A. pubescens* grows in dry, cold, often shady places at higher elevations. There can be no doubt that these ecological differences between the two entities correspond to different inherent physiological tolerances. Only in intermediate habitats, therefore, will genotypes intermediate between *A. formosa* and *A. pubescens* be able to succeed (Anderson, 1948). The ecological differences between the two entities are correlated with differences in time of flowering. *Aquilegia formosa* blooms early and *A. pubescens* blooms somewhat later. Although some colonies of the red and white columbines in the Slate Creek and White Mt. areas are completely isolated seasonally, most of the colonies overlap in flowering time during a period of several days to a week or more. The two entities, furthermore, are pollinated by different kinds of animals. The nodding red flowers of *A. formosa*, which are adapted to visits by hummingbirds, are inaccessible and unattractive to the hawkmoths which pollinate *A. pubescens*, and contrariwise the hummingbirds cannot feed successfully on the long-spurred *pubescens* flowers and do not try to do so.

These barriers are not absolute. The ecological separation of *A. formosa* and *A. pubescens* breaks down wherever an intermediate habitat suitable for the growth of both types appears, as at Saddlebag Lake. The uniform environmental conditions in one locality stimulate a convergence in flowering time and thus abolish a good part of the seasonal isolation which prevails elsewhere. The chance flight of some pollen-collecting bee from a *formosa* flower to a *pubescens* flower or vice versa may then result in the production of some hybrid individuals. Since the absence of sterility in these hybrids does not lead to a general amalgamation of the two entities into one interbreeding population, it is logical to conclude that the entities are separated by some other barriers than sterility which affect the reproduction of the F_1 and later generation hybrids.

The fact that the hybrid colony comes to consist of some F_1 hybrids, some backcrosses, and a few conservative recombination types, in addition to the

original parents, but shows no tendency to approach the variation pattern of an experimental F_2 (see figs. 2 and 3D), is most significant in this regard, and can only mean that the reproduction of the first generation hybrids is restricted in some way. The nature of the restriction is not entirely a matter of conjecture. Random interbreeding within the hybrid population is opposed by the behavior of the pollinating animals, which favors instead the building up of backcross types in the direction of one parent or the other. The uniformity of the habitat relative to the potential ecological variability in the hybrid colony, moreover, results in a selection against radical departures from parental or intermediate types (Anderson, 1948). Various internal restrictions on recombination such as linkage, pleiotropy, and developmental correlation also help to make radical departures from the parental or intermediate types unlikely, especially in a population as small as the one at Saddlebag Lake (Anderson, 1948, 1949; Smith, 1950).

The differences in mode of pollination and vegetative ecology between *A. formosa* and *A. pubescens*, therefore, while not sufficient to preclude occasional hybridization, do prevent a free exchange of genes between those two entities either before or after such hybridization. Those isolating mechanisms, in short, divert initial hybridization into backcrossing and introgression, and prevent it from obliterating the distinctness of the entities.

There is some doubt whether the presence of hybrid sterility would have made much difference to the final outcome of hybridization between *A. formosa* and *A. pubescens*. The hybrid between *Helianthus annuus* and *H. Bolanderi*, as shown by Heiser (1949), is so sterile that it is impossible to obtain an F_2 generation of any size, and some of the F_1 individuals are completely sterile, yet introgressive hybridization between these two species has greatly modified their original characters and has enabled one of them, *H. Bolanderi*, to extend its range from the California foothills to the neighboring valleys and from serpentine soils to cultivated fields. There is no evidence that introgression between the interfertile units, *Aquilegia formosa* and *A. pubescens*, has had any such profound evolutionary effects. These considerations show that hybrid sterility is by no means a necessary component of the reproductive isolation between species.

THE SPECIES PROBLEM

If *Aquilegia formosa* and *A. pubescens* are members of different species, as concluded in the foregoing section, the question which next arises is the delimitation of the species to which they respectively belong. Inasmuch as morphological discontinuities have proven better criteria of species limits in *Aquilegia* than have the results of breeding experiments, this problem can be approached with some hope of success from the study of herbarium specimens. The discussion which follows is based largely on the recent and comprehensive monograph of *Aquilegia* by Munz (1946).

Aquilegia formosa belongs to a group of columbines with nodding, red and yellow flowers, short straight spurs and short petal blades, which, in various forms, ranges over a large area in North America. *Aquilegia formosa* ranges in western North America from Alaska to northern California. In

Idaho it intergrades with *A. flavescens*, which then extends through the northern Rocky Mountains south to Colorado and Utah. *Aquilegia formosa* intergrades by gradual stages with the variety *truncata* in northern California, which in turn passes into variety *pauciflora* in the high Sierra Nevada, into *A. eximia* in the Coast Ranges, into variety *hypolasia* in southern California and Baja California, and *A. Shockleyi* in the Mojave Desert and southern Nevada and Utah. The latter unit is replaced by *A. desertorum* in Arizona, *A. triternata* in the Chiricahua and Sandia Mountains of Arizona and New Mexico, and *A. elegantula* in Colorado, New Mexico and northern Mexico east to Nuevo Leon. *Aquilegia elegantula* approaches in morphological characters, as in geographical range, the variety *latiuscula* of *A. canadensis*, which extends from the Edwards Plateau in Texas north to Nebraska and Missouri. *Aquilegia canadensis* var. *hybrida* continues from northern Nebraska to Manitoba, and variety *coccinea* continues throughout a vast area in the eastern United States, to be replaced finally on the Atlantic seaboard by typical *A. canadensis* and in northern Florida by the variety *australis*. A southern representative of the group is *A. Skinneri* in the Sierra Madre of western Mexico from Sonora to Jalisco.

This group of entities is collectively separated from all the other columbines by major morphological discontinuities. No such discontinuities are found between the various constituent entities, which intergrade and in general replace one another in different geographic areas. These facts suggest that the group comprises a well defined species complex, perhaps even one polytypic species. The complex can be known by its oldest Latin name as the *Aquilegia canadensis* group.

Aquilegia pubescens belongs to a group of columbines with erect pale yellow flowers, long slender gently curving spurs, and long petal blades, which is confined to the central and southern Rocky Mountains and the mountain ranges of the Great Basin and southwestern deserts. The northernmost representatives of the group are *A. caerulea* var. *ochroleuca* in the Rocky Mountains from Idaho to Colorado, and variety *alpina* in Wyoming. These units merge into typical *A. caerulea* in Colorado and variety *pinetorum* in Utah and eastern Nevada. *Aquilegia caerulea* var. *pinetorum* is replaced by *A. micrantha* at lower elevations in the four-corners country of southern Utah and Colorado and northern Arizona and New Mexico. In Utah and Nevada *A. caerulea pinetorum* overlaps with *A. scopulorum*, which then extends farther west in Nevada, to be replaced finally in the southern Sierra Nevada of California by *A. pubescens*. At Grand Canyon *pinetorum* intergrades with *A. chrysantha*, which ranges from there through Arizona and New Mexico to Sonora and Chihuahua. *Aquilegia chrysantha* gives way in turn to *A. Chaplinei* in the Guadalupe Mountains of eastern New Mexico and western Texas, *A. Hinckleyana* in southwestern Texas, and the distinctive long-spurred *A. longissima* in southwestern Texas, Coahuila and Nuevo Leon.

This complex of intergrading forms is separated from all the other columbines by a prominent morphological hiatus and hence necessarily by reproductive barriers. Like the *Aquilegia canadensis* group, it has a great center of variability in the southwestern United States. The named taxonomic units

in the complex appear to represent geographical races of a single polytypic species. It is appropriate to refer to this complex of races by the name of its oldest described member as the *Aquilegia caerulea* group.

A third group of North American columbines has nodding blue or purple flowers with relatively short hooked spurs and long petal blades. These columbines are evidently related by their morphological characters to an Eurasian complex centering around *Aquilegia vulgaris* and its allies. That complex covers a very large area from western Europe to eastern Asia, and includes *A. vulgaris*, *transsilvanica*, *Amaliae*, *atrata*, *sibirica*, *glandulosa*, *pubiflora*, *olympica*, *Kareliniana*, *oxysepala*, *amurensis*, *flabellata*, and some other named entities. It continues in North America with *A. brevistyla* from Alaska to Manitoba and South Dakota, and *A. laramiensis* and *saximontana* in Wyoming and Colorado, and possibly also *A. Jonesii* in Alberta, Montana and Wyoming. It seems probable that these units form one natural species complex, the *Aquilegia vulgaris* group.

At least two other species groups may be recognized within the genus *Aquilegia*, both of which are confined to Eurasia. The first of these is the *Aquilegia alpina* group, which is in general similar to the *vulgaris* complex except for the possession of straight rather than hooked spurs. The *alpina* group ranges from Spain through Europe and Asia to Japan, and is thus sympatric with the *vulgaris* complex over a wide area. It is represented in Europe by *A. alpina*, *Kitabelii*, *Einseleana*, *thalictrifolia*, *Bertolonii*, *pyrenaica*, *nevadensis*, and some others, and in Asia by *A. fragrans*, *Moorcroftiana*, *viridiflora*, *Buergeriana*, *Rockii*, and other types.

Aquilegia ecalcarata Maxim. with erect or nodding, purple or white, spurless flowers and prominent petal blades probably forms a separate species by itself. This central Asian species, which represents the most primitive type of columbine extant, is connected with the *alpina* and *vulgaris* groups by a series of transitional forms, such as *A. nivalis* and *A. parviflora*.

The genus *Aquilegia* consists, in summary, of five main species complexes. The most primitive of these, *A. ecalcarata* of central Asia, is related on the one hand to *Isopyrum*, and on the other to the Eurasian columbines belonging to the *alpina* and *vulgaris* groups. The *vulgaris* complex has extended into North America but has not attained a rich development on that continent. Most of the North American columbines belong to the *canadensis* and *caerulea* groups.

These species complexes all belong to the same life form, being long-lived, heavily-rooted perennial herbs. While ecological differences exist between the species groups, they are not in general of a greater order of magnitude than those which exist within a complex. The *Aquilegia canadensis* group, for example, includes races fitted for life in such varied habitats as are offered in the southeastern United States, Alaska, and the Mojave Desert; these ecological differences are as extreme as any separating the major species groups. Cytological differences have not been found in the columbines, inasmuch as all species, like the related genus *Isopyrum*, have seven pairs of homologous chromosomes (Gregory, 1941; Skalinska, 1935). Genetic differences of the sort that lead to incompatibility between the species or sterility and inviability

in the hybrids, while not entirely absent in *Aquilegia*, are rare and weakly developed (Anderson and Schafer, 1931, 1933; Skalinska, 1935; Clausen, Keck and Hiesey, 1945). (Sterility of F_1 s and loss of vigor and fertility in F_2 have so far been found chiefly in some, but not all, crosses of the Eurasian *vulgaris* group by the American *canadensis* or *caerulea* groups.)

The principal, and perhaps the most significant, differences between the species complexes of *Aquilegia* are therefore the differences in their flowers.

The differences in floral structure are significant because they adapt the species groups to pollination by different kinds of animals. The flowers of *Isopyrum* and *Aquilegia ecalcarata* are probably pollinated promiscuously by short-tongued bees and flies. The author has observed small flies visiting and pollinating *Isopyrum occidentale* in the Mount Hamilton Range of California. The development of spurs in the flowers of the *A. alpina* group has probably enabled that species complex to be pollinated somewhat more efficiently by bees. The longer, hooked spurs of the *A. vulgaris* group represent a further specialization for pollination by the longest-tongued bumblebees (Müller, 1883). Some Eurasian columbine, emigrating to North America, adapted itself to hummingbird pollination, and thereby became the progenitor of the *A. canadensis* group. Still another group, the *A. caerulea* complex, was differentiated in North America in response to moth pollination. The evolution of *Aquilegia* is therefore essentially the evolution of different pollination systems.

The differences in method of pollination enable the species groups to grow together with only a limited amount of hybridization or with no interbreeding at all. *Aquilegia vulgaris* interplanted with members of the *alpina*, *canadensis* and *caerulea* groups in England and open pollinated by bees thus remained completely isolated from the other species. One hundred and nineteen progeny grown from a double recessive plant of *A. vulgaris* in this plot consisted of 100 selfs, 19 outcrosses within the line, and no hybrids (Anderson and Schafer, 1933). The floral isolation between the *canadensis* and *caerulea* groups in California has already been described.

The combination of great morphological diversity with a high degree of interfertility in *Aquilegia* has often been regarded as anomalous and remarkable (Gregory, 1941; etc.). Increased knowledge about the nature of floral isolating mechanisms suggests that this combination of morphological differences and genetic interfertility, far from being unusual, may be due to a direct correlation. The morphological characters distinguishing the species groups comprise a series of floral mechanisms which adapt them to different pollinating animals. The success of the reproductive isolation which is thereby attained at the stage of pollination may enable the species to evolve without acquiring special sterility or incompatibility barriers.

The columbines are by no means unique in their weak development of sterility barriers. Orchids with very different flower forms can often be crossed with ease to yield fertile hybrids. The genus *Nigritella* is thus wholly or partially interfertile with eight other genera (Gustafsson, 1947), and many other fertile intergeneric hybrids have been produced in this family. Recent work on *Phaseolus* at the Carnegie Institution of Washington (Oliver Norwell,

unpubl.) has shown that species placed in widely different taxonomic sections and subgenera form fertile hybrids. Hybrids between morphologically dissimilar species of *Antirrhinum*, *Delphinium*, *Penstemon*, and cactus are likewise frequently fertile (Baur, 1930; Mather, 1947; Epling and Lewis, unpubl.; Lenz, unpubl.). These are all groups of plants in which a high development of floral isolating mechanisms is associated with a retarded development of sterility and incompatibility barriers.

SOME FIELD PROBLEMS

A great many interesting problems await the student of natural hybridization in the columbines. The purpose of the final section of this paper is to mention some areas where natural hybrids might be expected to occur.

The respective ranges of the *Aquilegia canadensis* group and the *A. caerulea* group in western North America are shown in fig. 4. The distribution areas have been mapped from the available distribution records, and are subject to extensions and corrections with future field studies. The map shows a wide area of overlap in the ranges of the two species complexes, which suggests at once that the possibilities of hybridization may be numerous and varied. An examination of the specimens representative of the two complexes in the herbaria of the Rancho Santa Ana Botanic Garden, Pomona College, Stanford University, and University of California yielded evidence of several hybrid colonies, which have been located on the map by crosses.

The colonies are from west to east: (1) Saddlebag Lake, Mono County, California; *A. formosa* var. *truncata* × *A. pubescens*; (V. Grant 8988, RSA). (2) Center Basin, Tulare County, California; *A. formosa* var. *truncata* × *A. pubescens*; (P. A. Munz 12561, RSA). (3) Charleston Mountains, Clark County, Nevada; probably *A. Shockleyi* × *A. scopulorum*; (I. W. Clokey 7094, Pom). (4) Shingle Creek, White Pine County, Nevada; probably *A. formosa* × *A. caerulea* var. *pinetorum*; (R. M. Bond, 87, UC). (5) Little Cottonwood Canyon and Alta, Wasatch Mountains, Utah; *A. flavescens* × *A. caerulea*; (M. Jones (1904 and 1898), Pom).

Population studies in these and other areas where the *A. canadensis* and *A. caerulea* groups meet and hybridize would be very rewarding.

The range of the whole *A. canadensis* group is plotted against the range of the *A. vulgaris* group in North America in another map (fig. 5). The map shows that opportunities for hybridization between these two species exist and are probably realized in various localities, as between *A. flavescens* and *A. brevistyla* in Jasper Park, Alberta (Munz, 1946, p. 102), and between *A. canadensis* var. *hybrida* and *A. brevistyla* in southern Manitoba and western South Dakota (op. cit., p. 122). An investigation of the relationships of these two species groups in the field would be most valuable.

To complete the hybrid triangle of North American columbines, the right-hand map in fig. 4 shows the geographical relationships of the *A. caerulea* group and the *A. vulgaris* group. Hybridization between these two species would be possible in the Rocky Mountains, and, if the pale blue color in the flowers of *A. caerulea* and *A. scopulorum* in this area is any criterion, probably does occur. Population studies are definitely needed in this region.

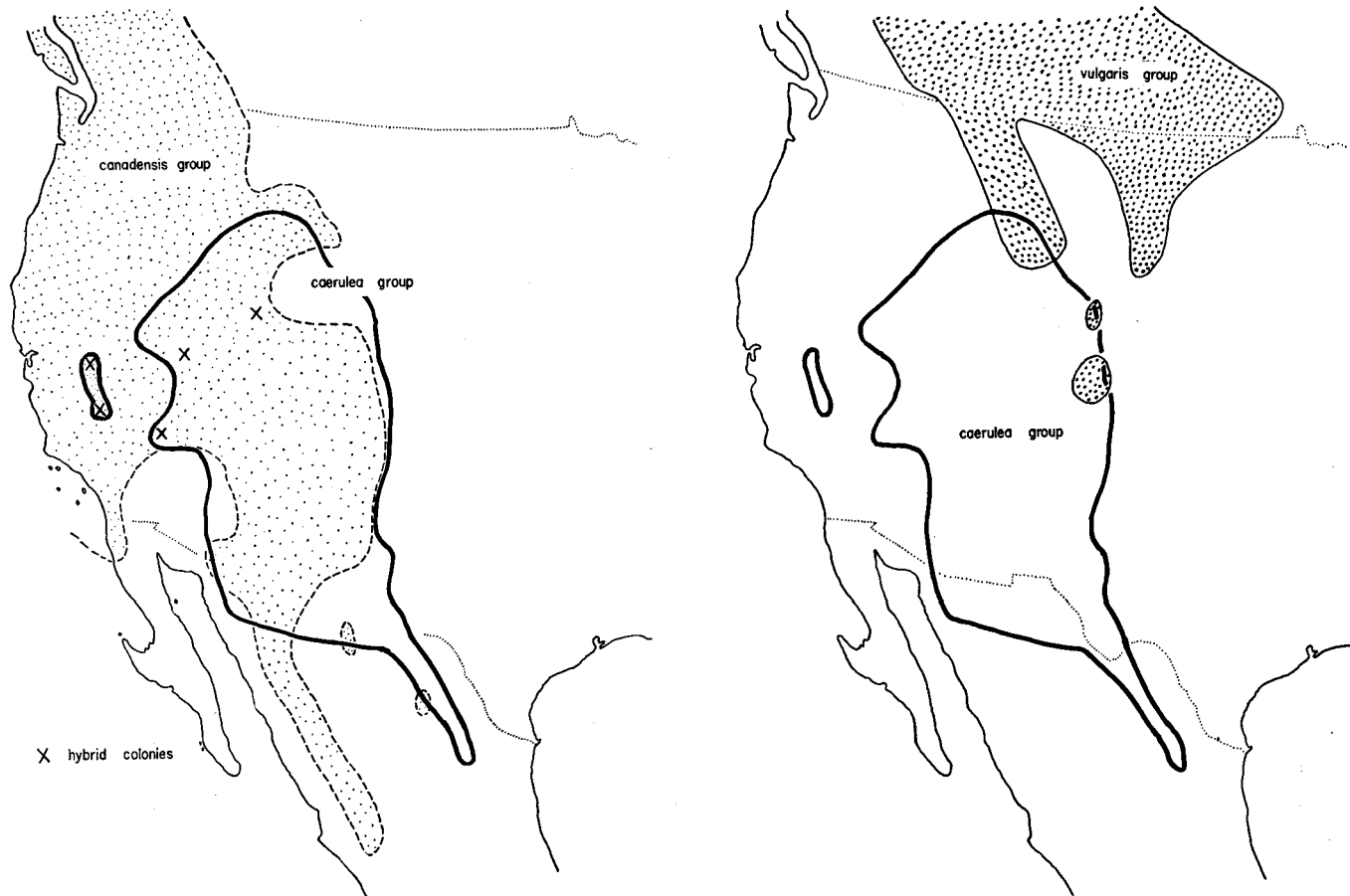


Figure 4. Maps of western North America comparing the distribution areas of the *Aquilegia caerulea* and *A. canadensis* groups (left) and the *A. caerulea* and *A. vulgaris* groups (right).

Hybridization between the *A. vulgaris* group and the *A. alpina* group, finally, probably occurs sporadically throughout Eurasia from Japan to the Alps, and poses numerous problems for botanists resident in that great continent.

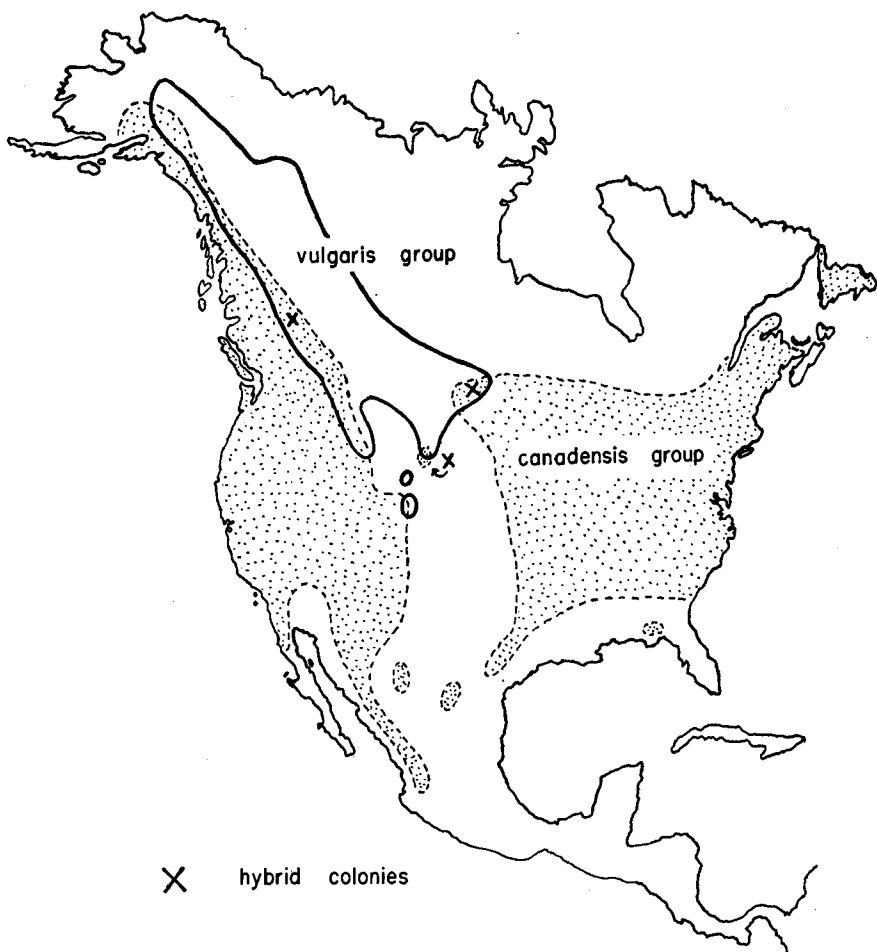


Figure 5. Map of North America comparing the distribution areas of the *Aquilegia canadensis* and *A. vulgaris* groups.

Beyond the discovery and the analysis of the hybrid colonies lies the really important question. How far do the effects of hybridization travel outside the immediate area of contact between the hybridizing species? It is where the effects of natural hybridization are imperceptible, according to Anderson (1949), and not where they are conspicuous and easily detected, that they have the greatest biological significance.

CONCLUSIONS

A study was made of reproductive isolation and natural hybridization between two interfertile species of columbine, *Aquilegia formosa* and *A. pubescens*, which occur together at high elevations in the southern Sierra Nevada of California. These two species are isolated mainly by differences in mode of pollination, *A. formosa* being pollinated by hummingbirds and *A. pubescens* by hawkmoths. The structure of the flowers and the behavior of the normal pollinators are opposed to interspecific pollinating visits. The hybrid colonies of columbines which occur in the Sierra Nevada are believed to owe their existence to the chance interspecific visits of pollen-collecting bumblebees. Random interbreeding does not occur within a hybrid colony, because the pollinating visits of the hummingbirds are confined to the *formosa* plants and the hybrid intermediates, and the visits of the hawkmoths to the *pubescens* plants and the hybrids. This system of cross-pollination, reinforced by selection against radical departures from the parental or intermediate types, has the effect of turning the initial hybridization into backcrossing and introgression. The floral isolating mechanism thus operates both before and after hybridization to preserve the distinctness of the two species.

The available evidence indicates that the most important species-separating barriers in the genus *Aquilegia* as a whole may be differences in mode of pollination. An examination of the morphological characters and geographical distribution of the 124 taxonomic species and varieties recognized in this genus suggests that they fall into about five species complexes which correspond to five different pollination systems. The primitive *Aquilegia ecalcarata* of Asia is probably pollinated by miscellaneous short-tongued bees and flies; the Eurasian *A. alpina* group is probably pollinated by bees; the Eurasian and North American *A. vulgaris* group is pollinated by long-tongued bumblebees; the North American *A. canadensis* group, which includes *A. formosa*, is pollinated by hummingbirds; and the North American *A. caerulea* group, which includes *A. pubescens*, is pollinated by hawkmoths. The reproductive isolation between these species complexes, which is necessary to maintain the discontinuities in morphological characters even despite frequent hybridization, is due not to hybrid sterility, but to major differences in method of pollination accompanied by differences in vegetative ecology. In the columbines, as in *Delphinium*, *Phaseolus*, *Antirrhinum*, *Penstemon*, and some orchids, a high development of floral isolating mechanisms is associated with a retarded development of incompatibility and sterility barriers.

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