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FLORAL NECTAR SPURS AND DIVERSIFICATION

SCOTT A. HODGES[1]

Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, California 93106

Key innovations are thought to be especially important adaptations that confer the ability to utilize resources in a novel manner and may therefore allow taxa to diversify. Here I review the data indicating that the evolution of floral nectar spurs represents a key innovation in *Aquilegia* and many additional groups. *Aquilegia* has apparently radiated recently and this radiation is closely associated with the evolution of nectar spurs. Numerous studies support the hypothesis that nectar spur morphology is important in plant reproduction and may provide a prezygotic reproductive isolating mechanism via differential pollinator visitation. The evolution of nectar spurs is highly correlated with increased species diversity across multiple independent lineages and thus provides strong support for the key innovation hypothesis. Previous studies have suggested that the evolution of nectar spurs may have been due to a simple genetic change in *Aquilegia*. I review these data and point out several cautions to this conclusion. Finally, I suggest that future research to determine the genetic basis for the development of nectar spurs will lead to especially interesting insights to the evolutionary origin of morphological novelties.

Introduction

The evolution of particular features of organisms has been considered a "key innovation" because it facilitates the transition to a new adaptive zone by conferring the ability to utilize resources in a novel manner (Simpson 1953). Occupation of a new adaptive zone may allow taxa to diversify, and thus the concept of key innovations is often used to explain clades that have particularly high species diversity. However, critical identification of key innovations that have resulted in species diversification is difficult (Cracraft 1990). Often the defining features of highly speciose groups are suggested as key innovations, resulting in a circular argument that these key innovations are associated with a highly diverse clade, yet the high diversity of the clade was used to identify the trait. In addition, it is often unclear how proposed key innovations can affect changes in diversity. Differences in diversity arise through differences in the rates of speciation and/or extinction, and therefore a causal link between a key innovation and these processes needs to be established (Cracraft 1990; Heard and Hauser 1995). Furthermore, large differences in species numbers can arise simply through stochastic processes (Raup et al. 1973; Slowinski and Guyer 1989, 1993), suggesting that key innovation hypotheses must be critically evaluated and statistically tested for changes in rates of diversification.

Phylogenetic analyses are essential for the identification and testing of key innovation hypotheses. Through these analyses it is possible to identify radiations of taxa that may be due to the acquisition of a key innovation. When coupled with field studies of the current influence of traits on processes important in diversification, phylogenetic analyses can provide broad support for key innovation hypotheses. In addition, phylogenetic analyses are essential for the identification of sister-group relationships and the robust testing of these hypotheses. Here I review the data (see also Hodges 1997) that have been used to hypothesize that the columbine genus, *Aquilegia,* experienced a recent and rapid radiation of taxa, that the development of floral nectar spurs is likely to be the key innovation that is responsible for this radiation, and that additional taxa that have nectar spurs may have independently experienced similar patterns of diversification. I then discuss the genetic basis of nectar spur development and possible future avenues of research.

Rapid radiation in *Aquilegia* and the evolution of nectar spurs

Within the columbine genus, *Aquilegia,* there is great diversity at the species level with respect to floral morphology and color, and these differences correspond to different pollination syndromes (Munz 1946; Grant 1952). *Aquilegia* species also occupy diverse habitats with some species restricted to high alpine environments and others to desert springs. Notwithstanding this diversity in both ecology and morphology, species in the genus are largely interfertile (Prazmo 1965*a,* 1965*b;* Taylor 1967). The high level of interfertility led some early workers to suggest that the genus was of recent origin (Clausen et al. 1945). However, other investigators (Stebbins 1950; Prazmo 1965*b;* Grant 1994*a*) have hypothesized that the genus is of at least mid-Tertiary age based largely on its widespread distribution in the Northern Hemisphere.

Phylogenetic analyses can establish whether lineages have radiated rapidly (Hodges and Arnold 1994*a*). For instance, a recent radiation will result in low levels of sequence divergence among species as compared to closely related groups that have not radiated rapidly. Among species of *Aquilegia,* levels of sequence divergence are very low compared with the levels of sequence divergence in the closely related genera *Isopyrum* and *Thalictrum* (fig. 1; Hodges and Arnold 1994*a*). Low nucleotide variation in *Aquilegia* could be explained by either a rapid radiation or a

[1]E-mail hodges@lifesci.ucsb.edu. Reprints available from the author.

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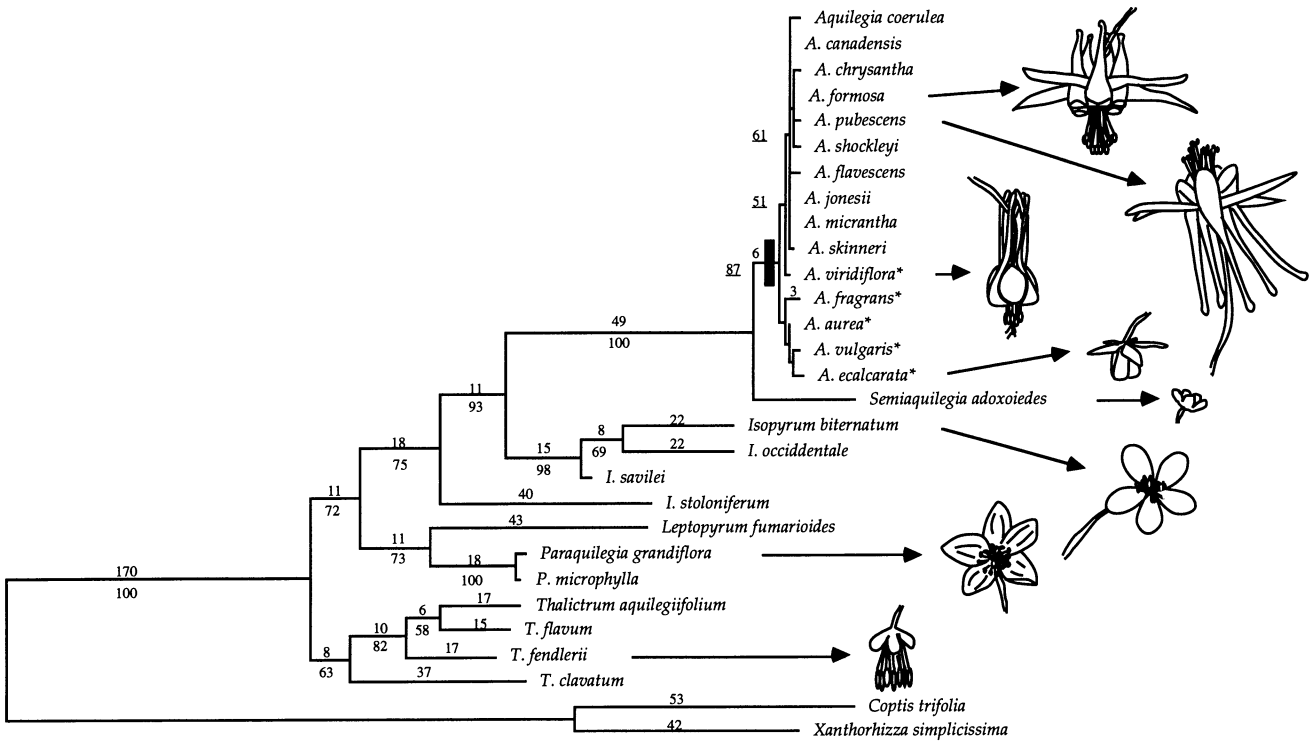

Fig. 1 Phylogram from a maximum parsimony analysis (Swofford 1993) DNA sequence data (ITS and cpDNA; Hodges and Arnold 1994, 1995) for *Aquilegia* and its close relatives. The single most parsimonious tree is depicted with percent bootstrap values (≥50%, 100 replicates) below or underlined and to the left of branches. Inferred branch lengths are indicated above branches. Line drawings of several of the taxa are indicated to the right of the phylogram (not to scale). The black bar represents the most parsimonious branch for the evolution of nectar spurs. Asterisks indicate *Aquilegia* species found in the Old World. Redrawn from Hodges and Arnold (1994, 1995).

drastic reduction in the rate of nucleotide substitutions. Reduction in nucleotide substitutions appears to be an unlikely explanation because the overall branch lengths for the columbine clade are not shorter than the branch lengths for their close relatives (fig. 1; Hodges and Arnold 1994*a*). Furthermore, this pattern of comparatively low sequence variation in *Aquilegia* was found in both a nuclear DNA region (the internally transcribed spacer [ITS] regions of the rDNA) and a chloroplast DNA region (the spacer between the *atp*B and *rbc*L genes; Hodges and Arnold 1994*a*). Similar patterns of low sequence variation in both a nuclear and chloroplast DNA region may indicate that low nucleotide variation is not an anomaly of a particular gene region but is indicative of a genome-wide phenomenon. Thus, it appears that *Aquilegia* experienced a rapid and recent radiation.

The radiation of *Aquilegia* is likely to have occurred via a key innovation rather than by invasion of a newly formed habitat with few competing species. The close relatives of *Aquilegia, Isopyrum* and *Thalictrum,* have a similar broad distribution. As such, *Aquilegia* does not occupy a geographic range that is substantially different from their close relatives that show no signs of having experienced a rapid radiation (fig. 1; Hodges and Arnold 1994*a*). Therefore, it does not appear that *Aquilegia* has dispersed into a new habitat that its close relatives were unable to invade.

Aquilegia species clearly differ from their close relatives in their mode of pollination. The genera *Isopyrum, Paraquilegia,* and *Thalictrum* all lack obvious morphological features for pollination by specific animals (fig. 1). The relatives of *Aquilegia* have open radiate flowers that would not restrict visitation or are wind pollinated as in many species of *Thalictrum.* However, in *Aquilegia,* floral morphology associated with different pollinators varies widely among species. Spurs in different species have varying lengths and orientations, they may be straight or curled, and they also vary in their coloration, including blue, purple, white, yellow, and red species (Munz 1946). Thus Hodges and Arnold (1994*a*) proposed that the evolution of nectar spurs was a key innovation for this group that allowed specialization to different pollinators and promoted diversification. Subsequent analyses incorporating two nonspurred taxa, *Aquilegia ecalcarata* and *Semiaquilegia adoxoides,* that were unavailable for the initial study, reinforced this conclusion. Inclusion of the sequences for ITS and the *atp*B-*rbc*L spacer for these species with the original sequences resulted in a single tree (fig. 1; Hodges and Arnold 1995; Hodges 1997). The relationship of *A. ecalcarata* could not be resolved among other European and Asian *Aquilegia,* but *S. adoxoides* appears to be the sister taxon of *Aquilegia* and quite closely related. Thus, the most parsimo-

nious placement of the evolution of nectar spurs is restricted to the short branch leading to *Aquilegia* (fig. 1), suggesting a close temporal link between the evolution of nectar spurs and the rapid diversification of *Aquilegia* (Hodges and Arnold 1995; Hodges 1997). Though the pattern of diversification in *Aquilegia* is suggestive of an increase in the rate of diversification within the columbine clade, several theoretical studies have shown that large differences in diversity between clades can occur purely through stochastic processes (Raup et al. 1973; Slowinski and Guyer 1989, 1993). To test whether the diversity in *Aquilegia* is greater than that expected under a random model of diversification, Hodges and Arnold (1995) used the maximum likelihood approach developed by Sanderson and Donoghue (1994). This method is based on a model of random speciation only and utilizes the diversities of three branches of a clade: one outgroup and two sister taxa that form the ingroup. The maximum likelihood model then determines which models of changes in diversification best fit the species diversities of the clades (Sanderson and Donoghue 1994). To fit a simple key innovation hypothesis, the branches delineating taxa that possess a suspected key innovation will also show changes in diversification. Using *Semiaquilegia* (1 sp.) as the sister taxon to *Aquilegia* and the Old World (47 spp.) and the New World (23 spp.) *Aquilegia* as the internal sister groups (fig. 1), Hodges and Arnold (1995) found that the simple models that were incompatible with a key innovation hypothesis could all be rejected. Thus only models compatible with a key innovation hypothesis remained, supporting the hypothesis of a correlation between the evolution of nectar spurs and the increased diversification in *Aquilegia.*

Recently, additional approaches have been proposed to test for changes in diversification rates in phylogenetic trees. Wollenberg et al. (1996) proposed a method utilizing branching patterns to test for ancient or recent changes in diversification. The method relies on a stochastic model of lineage bifurcation and extinction, such that a cumulative distribution of branching times could be calculated and then compared with the distributions found from empirical trees. Thus, empirical trees that have significant differences in branching patterns from the random trees indicate changes in diversification rates. Utilizing the sequence data for *Aquilegia* (Hodges and Arnold 1994*a,* 1995), Wollenberg et al. (1996) found that indeed, *Aquilegia* fit a model of rapid, recent diversification. In addition, Wollenberg et al. (1996) found support for a change in diversification between *Semiaquilegia* and *Aquilegia* but not within *Aquilegia,* supporting a change in diversification associated with the timing of the evolution of nectar spurs.

Nectar spurs and diversification, a causal link?

While the above analyses clearly indicate that *Aquilegia* has gone through a rapid and recent radiation, determining whether a specific feature may be causal in promoting the radiation is much more difficult. This difficulty arises because there can be multiple characters that are correlated with the shift in diversification rate. Two types of information are important in addressing whether a specific feature may result in increased diversification rates. First, it is essential to understand how a particular character may directly influence diversification by either increasing speciation rates and/or decreasing extinction rates. Second, correlations across multiple taxa that have acquired the same or similar traits with increased diversification should provide strong support for a key innovation hypothesis.

Can nectar spur morphology influence speciation and/or extinction rates? Most species concepts incorporate the necessity of reproductive isolation (Dobzhansky 1937; Mayr 1942; Grant 1963). Therefore, characters that can promote reproductive isolation may increase speciation and thus diversification rates. Because the length, shape, color, and orientation of nectar spurs can influence the types of pollinators that visit flowers, they are closely linked with reproduction and reproductive isolation. Thus, if species differ in their floral morphology and consequently differ in the types of visitors that pollinate their flowers, then even if the species come into secondary contact, selection for divergent flower morphologies via pollinator preferences may provide a prezygotic reproductive isolating mechanism and prevent the taxa from merging (Grant 1952; Hodges and Arnold 1994*a,* 1995).

Numerous studies show that nectar spur characters are highly correlated with the types of pollinators that visit plants and the reproductive success of individual plants. In the orchid genus *Plantanthera,* Nilsson (1988) showed that experimentally reducing the length of nectar spurs had a pronounced effect on both the insertion and removal of pollinia by pollinators as well as fruit set. In a separate orchid genus, *Disa,* Johnson and Steiner (1997) showed that long-spurred populations are pollinated by long-tongued flies and that artificially reducing spur length significantly reduced pollen receipt and fruit set. Thus, spur length in both of these genera has been found to directly influence reproduction.

Among populations of a species and among species in a genus, spur morphology is highly correlated with pollinator morphology. Again, in *Disa* (Johnson and Steiner 1997), populations vary widely in nectar spur morphology (mean spur length varies from 32 mm to 72 mm). These differences in spur length are highly correlated with the tongue lengths of the flies that visit them. Similar correlations of spur length and pollinator morphology have been reported for *Diasia* (Steiner and Whitehead 1990, 1991), *Plantanthera* (Robertson and Wyatt 1990), *Aconitum* (Brink 1980), and *Aquilegia* (Miller 1981), encompassing a spectrum of pollinators (flies, butterflies, bees, bees and hawkmoths, respectively). These studies suggest that pollinators can strongly influence spur morphology (and vice ver-

sa) and that in doing so, may provide a mechanism for reproductive isolation if these taxa come into secondary contact.

The occurrence of hybrid zones can be used to test whether floral traits influence reproductive isolation. If floral traits of hybridizing taxa do not influence pollinator behavior, then these traits will introgress across the hybrid zone to a greater extent than traits that reduce fitness in hybrids. Within *Aquilegia,* there are numerous observations of hybrid zones between species with different floral morphologies (Grant 1952; Whitemore 1997). Among these hybrid zones, those between *Aquilegia formosa* and *A. pubescens* have been most extensively studied (Grant 1952; Chase and Raven 1975; Hodges and Arnold 1994*b*). *Aquilegia formosa* has short, pendent, red-spurred flowers predominately visited by hummingbirds, while *A. pubescens* has long, upright, white or yellow-spurred flowers predominately visited by hawkmoths (Grant 1952; Grant 1993; Grant 1994*b*), though Chase and Raven (1975) have suggested that habitat type may be more important in promoting reproductive isolation between these two species.

To test whether floral morphology is important in reproductive isolation between *A. formosa* and *A. pubescens,* Hodges and Arnold (1994*b*) measured the shapes of clines for floral features and molecular markers between these species. Cline shape can be characterized as the rate of transition between character states across a physical distance. Because the steepness of clines depends on the strength of natural selection and gene flow, differences among characters in their cline shape can be used to infer how selection is acting in a hybrid zone (Endler 1986; Barton and Hewitt 1989). Steep clines indicate that selection is preventing the spread of a character across a hybrid zone (and thus that it may be important in reproductive isolation), while broad clines indicate neutral or weakly selected traits or traits that are selected to transverse the hybrid zone. Hodges and Arnold (1994*b*) found that most floral characters (including spur length and orientation) formed sharp clines while random regions of DNA (randomly amplified polymorphic DNA [RAPDs]) that are presumably neutral with respect to plant fitness formed broader clines (Hodges and Arnold 1994*b*). Thus, these data are consistent with the hypothesis that spur morphology may promote different pollinator visitation to *A. formosa* and *A. pubescens* and thereby provide a mechanism for reproductive isolation between these species. Clearly, spurs do not confer complete reproductive isolation, or else hybrids would not exist at all. Grant (1952) suggested that hybrid zones are started by occasional bee pollination between the species. Even if the major pollinators cause some hybridization, the sharpness of the clines in floral morphology suggest that pollinators are likely to predominantly discriminate between the species.

Table 1

FAMILIES WITH TAXA POSSESSING FLORAL NECTAR SPURS

Family	Taxa with spurs (or examples)
Balsaminaceae	···
Campanulaceae	*Heterotoma*
Caprifoliaceae	Some *Lonicera*
Fumaraceae	···
Genianaceae	*Halenia*
Geraniaceae	*Pelargonium*
Lentibulariaceae	···
Leguminosae	Ex *Amherstia, Bauhinia*
Orichidaceae	Ex *Angraecum, Habenaria*
Ranunculaceae	*Aconitum, Delphinium Aquilegia*
Scrophulariaceae	Ex *Chaenorhinum, Cymbalaria, Diascia, Kickxia, Linaria, Nuttalanthus*
Tropaeolaceae	···
Valarianaceae	*Centranthus*
Violaceae	*Anchietea, Corynostylis Noisettia, Viola*
Vochysiaceae	Except *Amphilochia, Euphronia*

Note. If taxa column is blank, then family is defined by the presence of spurs.

Comparative studies of nectar spurs and diversification

Many groups of flowering plants possess nectar spurs (table 1), and there have likely been many independent origins of the trait, a hypothesis supported both by large phylogenetic distances between groups possessing nectar spurs and by the different developmental origins of spurs. For instance, in *Aquilegia,* the spurs are found on the petals, while in *Delphinium* and *Aconitum* (distantly related within the Ranunculaceae; Hoot 1991, 1995), the more complex spurs are a product of two petals and a sepal. Likewise, spurs in the Balsaminaceae (e.g., *Impatiens*) are in the sepal whorl, while in the Lentibulariaceae (e.g., *Utricularia*), spurs are in the petal whorl.

Based on the independent origins of spurs in different plant groups, a correlation between the origin of nectar spurs and species diversification can be tested. Once multiple independent origins of a suspected key innovation have been identified, identification of their sister group is necessary for tests of differential diversification. Sister groups are, by definition, the same age, and therefore any differences between them in species numbers are due to differences in the rates of speciation and/or extinction. Therefore, one simple test for a correlation between a trait and diversity is the sign test; simply counting the number of groups where the lineage with the key innovation has more species than its sister group (e.g., Mitter et al. 1988). For the eight lineages in which floral nectar spurs have evolved and sister groups can be identified (table 2), seven have more species in the clade with floral spurs (sign test, $P < 0.05$). Thus, there is a significant trend for more species to occur in lineages possessing nectar spurs.

In addition to the sign test, more refined models have been developed because large differences in species numbers between sister groups can arise through random processes, and it has been shown that these

Table 2

NUMBER OF SPECIES IN GROUPS THAT HAVE INDEPENDENTLY EVOLVED FLORAL NECTAR SPURS AND THEIR INFERRED SISTER GROUPS (see references)

Spurred taxa	Nonspurred sister taxa	No. of spurred spp.: nonspurred spp.	*P*	References
Aquilegia	*Semiaquilegia*	70:1	0.014	Hodges and Arnold 1995
Delphinium, Aconitum	*Nigella*	350:14	0.039	Hoot 1991, 1995
	or	or	or	
Delphinium, Aconitum	*Nigella, Actaea, Cimicfuga*	350:37	0.095	Johansson and Jansen 1993
Fumariaceae	*Hypercoum*	450:15	0.032	Hoot and Crane 1995
Tropaeolaceae	Akaniaceae, Bretschneideraceae	88:2	0.022	Chase et al. 1993; Rodman et al. 1993
Anchiectia, Corynostylis	*Agatea*	12:1	0.083	Hodges, Ballard, Arnold, and Chase, unpublished data
Noisettia, Viola	Subset of *Hybanthus*	401:<150	?	Hodges, Ballard, Arnold, and Chase, unpublished data
Lentibulariaceae	Byblidaceae	245:2	0.008	Olmstead et al. 1993; Bremer et al. 1994
Pelargonium	*Geranium, Erodium, Monsonia, Sarcocaulon*	280:399	0.588	Price and Palmer 1993

differences can be quite large (Slowinski and Guyer 1989, 1993; Sanderson and Donoghue 1994). Therefore, to determine if differences in species numbers between sister taxa are due to significant changes in diversification rates, random models of speciation and extinction should be utilized. Slowinski and Guyer (1993) proposed a method to test for increased diversification between sister taxa and showed that a single clade of n species with a basal split into two clades of r species and $n-r$ species, all combinations of r and $n-r$ species respectively, are equally likely. Slowinski and Guyer (1993) then showed that the probability of observing a given species diversity in a clade with a proposed key innovation or an even greater difference can be calculated as $(n-r)/(n-1)$, where r is the number of species in the clade with the proposed key innovation and n is the total number of species in both clades. Six of the seven spurred taxa, where the diversity of the sister group has been identified (the number of species of *Hybanthus* that are sister to *Viola* and *Noisettia* is presently unknown), have significant or nearly significant deviations from the expected value (table 2). Thus, this test provides strong evidence that high species diversification is associated with the evolution of nectar spurs.

The pattern of increased diversity associated with the evolution of nectar spurs is striking. Few studies have been able to identify and test a key innovation hypothesis (Mitter et al. 1988; Farrell et al. 1991), and the strong correlation between the presence of nectar spurs and species diversity is exceptional. There are several reasons that such a strong correlation might not have been found, even if nectar spurs do in fact alter diversification patterns. First, if a sister group evolves a different key innovation, then no difference in diversity may be observed. Furthermore, a key innovation may only promote diversification in certain ecological settings (Simpson 1953). Alternatively, if the key innovation has recently evolved, differences in diversification may have not yet developed. Thus, the strong pattern between diversity and the evolution of nectar spurs is especially noteworthy. In the future, additional tests of the pattern of species diversification and the evolution of nectar spurs can be made as the sister taxa for additional groups with nectar spurs (table 1) are identified. In addition, the methods of Sanderson and Donoghue (1994) could be applied to test whether the change in diversification occurs on the branch containing the key innovation in each of these instances.

Genetics and development of nectar spurs

The outline of the studies above clearly suggests that the evolution of nectar spurs has led to increased diversification in *Aquilegia* and many other taxa of flowering plants. Thus, it would be especially interesting to understand the developmental basis of the formation of nectar spurs. Tepfer (1953) studied the later developmental pattern of nectar spurs in *Aquilegia formosa.* However, there are no detailed developmental comparative studies with nonspurred relatives. Such a study could shed light on the developmental events responsible for spur formation.

In *Aquilegia* and relatives, a comparison of floral development of spurred *Aquilegia* and the nonspurred *A. ecalcarata* and *Semiaquilegia adoxoides* would be especially informative. Comparative studies among these species could reveal at what developmental stage the morphology of spurred versus nonspurred taxa diverge. Such studies are important to identify the developmental stage where gene action may promote spur production and how the timing of spur development may constrain or promote its evolution in Angiosperms. For instance, the expansion of the nectar spur in *Aquilegia* occurs relatively late in floral development. Based on the late developmental timing, Gottlieb (1984) suggested that a major gene could be responsible for the evolution of spurs because it acts late in the differentiation of the flower and therefore would not affect earlier processes in floral develop-

ment. However, early in development, *Aquilegia* petals already have a cup shape that may be necessary for later development of the spur. A detailed study of petal development in spurred and nonspurred *Aquilegia* and relatives could reveal that the important developmental differences in spur development are established at an earlier time point.

Another important goal of future studies is to determine the genetic basis of spur development. The acquisition of spurs in *Aquilegia* has been used as one of the few examples of where the genetic basis of an adaptation has been investigated and that it may be due to a major gene (Gottlieb 1984; Orr and Coyne 1992). This assertion is based on the work of Prazmo (1965*a*), who made crosses between the spurless *A. ecalcarata* and several spurred species of *Aquilegia.* In all crosses, the F_1 progeny possessed spurs. The F_2 progeny predominantly segregated in ratios (spurred: spurless) indistinguishable from 3 : 1 (rarely 15 : 1), indicating that the presence of spurs was caused by the action of one or two genes. Furthermore, backcrosses to *A. ecalcarata* gave 1 : 1 ratios, again indicating a single gene character (Prazmo 1965*a*).

While the data for the genetics of spur development in *Aquilegia* are very intriguing, caution is necessary in the interpretation that one or two mutations were all that were needed for the evolution of spurs. First, the phylogenetic position of *A. ecalcarata* is unresolved within *Aquilegia* (bootstrap support <50%; fig. 1). Most authors have interpreted *A. ecalcarata* as primitive within the genus (Munz 1946; Grant 1952; Prazmo 1965*a,* 1965*b*), however, this assumption is largely based on the fact that this species lacks spurs. It is possible, though, that *A. ecalcarata* is derived within *Aquilegia* and that it represents a secondary loss of spurs. In fact, the single most parsimonious tree based on the DNA sequences data place *A. ecalcarata* as derived within the major European/Asian clade (fig. 1). If *A. ecalcarata* represents a secondary loss of spurs, then it could simply have had a mutation in a gene early in a cascade of many genes necessary for spur development. Such a mutation would appear as a major gene for spur development though many genetic changes could have been necessary for the evolutionary development of the spurred trait.

A second caution in the interpretation of the genetics of spur development in *Aquilegia* arises from the definition of a spur. Prazmo (1965*a*) notes that in F_2 populations derived from crosses between *A. ecalcarata* and spurred *Aquilegia,* the degree of spur development varies continuously among plants possessing spurs. Thus, the ratios of spurred: spurless plants found by Prazmo (1965*a*) are partially dependent on his definition of a spur. To what degree of protuberance from the base of a petal is the presence of a spur declared? Because categorizing plants as spurred/spurless must be at least partially subjective, divisions in support of simple Mendelian segregation patterns may be more likely.

Several types of future studies may resolve whether the development of spurs in *Aquilegia* is due largely to the action of a major gene or to many genes each with small effect. Resolving the number of genes involved in producing particular traits is possible by following the segregation of markers along chromosomes with the phenotypic value of individual plants (Lander and Botstein 1989). This method is referred to as quantitative trait locus (QTL) mapping. In such an analysis, individuals do not have to be classified into discrete categories, and with markers spanning the entire genome, the number of genomic regions and their phenotypic effects can be determined. QTL mapping is only beginning to be applied to natural species (e.g., Bradshaw et al. 1995) and promises to be a powerful technique in measuring the genetic basis of adaptive traits. Such an analysis of *A. ecalcarata* and spurred *Aquilegia* could be very informative for resolving the genetic basis of spur development in *Aquilegia* and should be quite feasible as the genome is small (ca. twice the size of *Arabidopsis;* Bennett and Smith 1982, 1991), and it is diploid with few chromosomes (n=7).

However, as noted above, the phylogenetic position of *A. ecalcarata* is crucial to the interpretation of the genetic basis of the evolution of nectar spurs in *Aquilegia.* Even if *A. ecalcarata* is found to be basal to all other *Aquilegia,* a hypothesis that the presence of spurs is ancestral and *A. ecalcarata* secondarily lost spurs would only require a single additional step to the most parsimonious explanation. Thus, the most direct evidence of the genetic basis of spur development may come from the analysis of independent mutant lines. There are several reports of individual mutant plants or populations of *Aquilegia* that lack nectar spurs. For instance, Eastwood described a population of spurless *Aquilegia* in southern Colorado as *Aquilegia ecalcarata* (Munz 1946). Apparently this was a small population of spurless *A. micrantha* (Munz 1946). In addition, spurless forms have been reported for *A. formosa, A. caerulea,* and *A. vulgaris* (Munz 1946). Thus, it appears that mutations causing the loss of spurs are relatively common. Assuming that these mutants are independent, then they could be used in a crossing program to determine if the mutants affect different genes. Crosses between mutant stocks can result in the reappearance of spurs suggesting complementation and that spurlessness arose through mutations in separate genes. Alternatively, comparative QTL mapping of spurs utilizing several mutant lines and the same spurred line would determine whether the same genomic regions were influencing spur development in all mutants.

Conclusions

The identification of key innovations may lead to especially interesting studies of the evolution of morphological novelties. The identification of specific features of organisms that influence patterns of diversity provides a strong impetus to understand the development of these features. Future studies on the devel-

opment of nectar spurs will be particularly fruitful in this regard. As indicated above, *Aquilegia* may provide a number of advantages for such studies due to the ability to cross species with widely divergent floral morphologies and the presence of multiple independent mutant lines. In addition, because nectar spurs have evolved independently in multiple groups of angiosperms, future comparative studies may elucidate whether similar developmental patterns are followed in different taxa and perhaps whether or not similar genes are involved.

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