

Spurring plant diversification: are floral nectar spurs a key innovation?

SCOTT A. HODGES¹ AND MICHAEL L. ARNOLD²

¹*Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93106, U.S.A.*

²*Department of Genetics, University of Georgia, Athens, GA 30602, U.S.A.*

SUMMARY

High levels of species diversity in taxonomic groups have often been explained by a key innovation. However, the difficulty in establishing a causal role between a proposed key innovation and increased species diversity, as well as in substantiating that diversity patterns are different from null models has led to major criticisms of key innovation hypotheses. Here we show that patterns of diversification within and among clades that have evolved floral nectar spurs strongly support the hypothesis that floral nectar spurs represent a key innovation. Both reproductive success and reproductive isolation can be influenced by simple changes in nectar spur morphology and the acquisition of nectar spurs in a wide array of plant groups is highly correlated with increased species diversity.

1. INTRODUCTION

A fundamental goal in biology is to understand the factors that influence organismal diversity (Darwin 1872; Mayr 1942; Simpson 1953). Key innovations are novel morphological or behavioural traits thought either to open new 'adaptive zones' (Simpson 1953) or to offer the ability to rapidly speciate after environmental change (Liem 1973). Thus, the evolution of a key innovation is thought to be a particularly important event affecting subsequent patterns of speciation and/or extinction. Despite the common use of key innovations as an explanation for clade-specific increases in diversity, the concept has been criticized as being essentially untestable (Cracraft 1990). First, demonstrating how a particular trait can cause rates of diversification to increase is often problematic (Cracraft 1990). Second, large differences in species numbers between sister taxa can be explained solely through stochastic processes (Raup *et al.* 1973; Slowinski & Guyer 1989, 1993). Here we consider these points in determining if floral nectar spurs are a key innovation, particularly in the columbine genus *Aquilegia*.

An underlying assumption of most species concepts is the necessity for reproductive isolation (Dobzhansky 1937; Mayr 1942; Grant 1963). Characters that can increase the likelihood of reproductive isolation may therefore increase rates of speciation. Nectar spur morphology is intimately tied to reproduction and can influence reproductive isolation. Spurs are tubular outgrowths of petals, or other floral parts, that increase the distance between the floral reward and the reproductive parts of the flower. Relatively simple differences in the length, shape, orientation, and colour of nectar spurs are associated with different pollinators

and affect reproduction. For instance, in orchid species, the length of an individual plant's spur strongly influences reproductive success (Nilsson 1988). In *Aquilegia caerulea*, variation among populations in the length and colour of spurs is correlated with variation in the types of pollinators visiting the plants (Miller 1981). Differences in spurs corresponding to different pollinators (hummingbirds versus hawkmoths) have also been shown to aid in reproductive isolation between two hybridizing *Aquilegia* species (Grant 1952; Hodges & Arnold 1994*b*). Similarly, in *Diascia*, variation among species in spur length is correlated with morphological variation in their bee pollinator (Steiner & Whitehead 1990; Steiner & Whitehead 1991). Together these studies suggest that differences in nectar spurs among populations of a species can facilitate reproductive isolation and possibly lead to increased speciation.

Among species of *Aquilegia*, there are low levels of DNA sequence divergence despite the widespread distribution of the genus, suggesting a recent radiation resulting from a key innovation (Hodges & Arnold 1994*a*). To determine if the evolution of nectar spurs was a key innovation for this genus we sought to temporally link the evolution of nectar spurs with an increase in diversification rate in the columbines. Using a molecular phylogenetic approach, we constructed phylogenies that included two spurless species, *Aquilegia ecalcarata* and *Semiaquilegia adoxoides*. These species have been suggested to be basal to the columbine clade (Munz 1946) and could thus reveal whether spurs evolved near the time of the radiation. Additionally, because nectar spurs have evolved independently in numerous lineages, we used null models to test if increased diversification is generally linked with the evolution of floral nectar spurs.

2. MATERIALS AND METHODS

Phylogenetic analyses were conducted using DNA sequences of the nuclear internally transcribed spacer region and the chloroplast intergenic spacer between the *rbcl* and *atpB* genes (Hodges & Arnold 1994a). Amplification and sequencing protocols have previously been described (Hodges & Arnold 1994a). Species analysed included all those used in our previous analysis (Hodges & Arnold 1994a) and in addition, two species that were unavailable at that time, *Aquilegia ecalcarata* and *Semiaquilegia adoxoides*. As previously described, *Coptis trifolia*, and *Xanthorrhiza simplicissima* were used to root the tree and PAUP (Swofford 1993) was used to analyse the data (Hodges & Arnold 1994a).

The pattern of diversification in *Aquilegia* was analysed by using Sanderson & Donoghue's maximum-likelihood method (Sanderson & Donoghue 1994). This method uses a random speciation model to test if observed patterns of diversity in a three-taxon phylogeny (two clades that share a proposed key innovation and their sister group) support a key innovation hypothesis. Within *Aquilegia* there are two distinct clades that predominately group the European and Asian species apart from the North American species (Hodges & Arnold 1994a); *Semiaquilegia* is the sister group for *Aquilegia* (see figure 1). We thus compared the diversity of *Semiaquilegia*, (one species) with the Old and New World columbine species (47 and 23 species, respectively).

In a survey of published and unpublished data we identified cladistic analyses that included taxa with nectar spurs. Because the tests of diversification patterns depend on the identification of sister taxa, we restricted our analysis to studies that probably contained representatives of all potential sister groups for each clade where nectar spurs have evolved. Once sister taxa were identified, we tested if the diversity of species was significantly greater in the spurred taxa compared with their non-spurred sister taxa using a model of random speciation and extinction and both a Wilcoxon sign test and Fisher's combined probability test (Slowinski & Guyer 1993) to test for the overall association between the evolution of nectar spurs and increased diversification. The number of species in each group was obtained from Mabberley (1993).

3. RESULTS

The phylogenetic reconstruction of *Aquilegia* and related taxa resulted in a single most parsimonious tree with *Semiaquilegia adoxoides* closely aligned and basal to the columbines whereas *Aquilegia ecalcarata* is placed within the basal clade of the columbines (see figure 1). The placement of the spurless *Semiaquilegia* indicates that the most parsimonious position for the evolution of nectar spurs is close to the columbine radiation (figure 1). This branch has relatively few inferred nucleotide changes compared to the branch connecting the *Aquilegia/Semiaquilegia* clade with the *Isopyrum* clade (six versus 49 inferred changes, figure 1). These data

suggest a temporal link between the evolution of nectar spurs and the columbine radiation.

To test if the diversification of *Aquilegia* matches a key innovation hypothesis, we used Sanderson & Donoghue's maximum-likelihood (ML) approach (Sanderson & Donoghue 1994). This method can exclude a key innovation hypothesis if either the model with no change in rate of diversification or a model with a change in only one of the basal lineages of the ingroup is supported. The one-parameter model, H_1^1 (H_j^k refers to the j th model within the class of models that has k parameters; Sanderson & Donoghue 1994) has no variation in rates of diversification while the two two-parameter models, H_2^2 and H_3^2 , have a change in diversification rate but only in one branch of the clade containing the key innovation (see figure 2). These models are rejected by the analysis (figure 2, $P \geq 0.95$). Both two-parameter models that are consistent with a key innovation hypothesis were not rejected by the analysis. Three-parameter models were not tested because two-parameter models are sufficient to describe the data (Sanderson & Donoghue 1994).

Significantly greater diversity than expected was detected in either four or five out of the six groups that have independently evolved nectar spurs (table 1). The relation was equivocal for *Delphinium* and *Aconitum* depending on the sister group identified. Using the sister group *Nigella*, identified by Hoot based on morphology (Hoot 1991) and DNA sequences of *rbcl*, *atpB*, and 18s rDNA (Hoot 1995), the relation is significant ($P = 0.039$) whereas using the sister group, *Nigella*, *Actaea*, and *Cimicifuga*, identified by Johansson & Jansen (1993) based on chloroplast DNA restriction site variation, the relation was not significant ($P = 0.095$). The single clear exception, *Pelargonium*, had fewer species in the spurred group (table 1). Five of the six groups had more species in the spurred clade than the non-spurred clade and thus a Wilcoxon sign test is nearly significant ($P = 0.059$). Regardless of the sister group used for *Delphinium* and *Aconitum*, Fisher's combined probability test (Slowinski & Guyer 1993) is highly significant ($\chi^2 = 38.48$ or 40.26 , d.f. = 12, $P < 0.001$).

4. DISCUSSION

The pattern of diversification in *Aquilegia*, along with its widespread distribution, suggest that it has undergone a rapid radiation because of a key innovation (Hodges & Arnold 1994a). Here we have shown that the evolution of nectar spurs is temporally linked with the increase in diversification of *Aquilegia* (figure 1) and that null models fail to reject a key innovation hypothesis (figure 2, table 1). These data strongly support a key innovation hypothesis for *Aquilegia* and, together with the general finding of increased species diversity in groups that have evolved nectar spurs (table 1), strongly support nectar spurs as an example of a key innovation.

Because nectar spur morphology is intimately tied to reproductive success and reproductive isolation, a causal link between the evolution of nectar spurs and

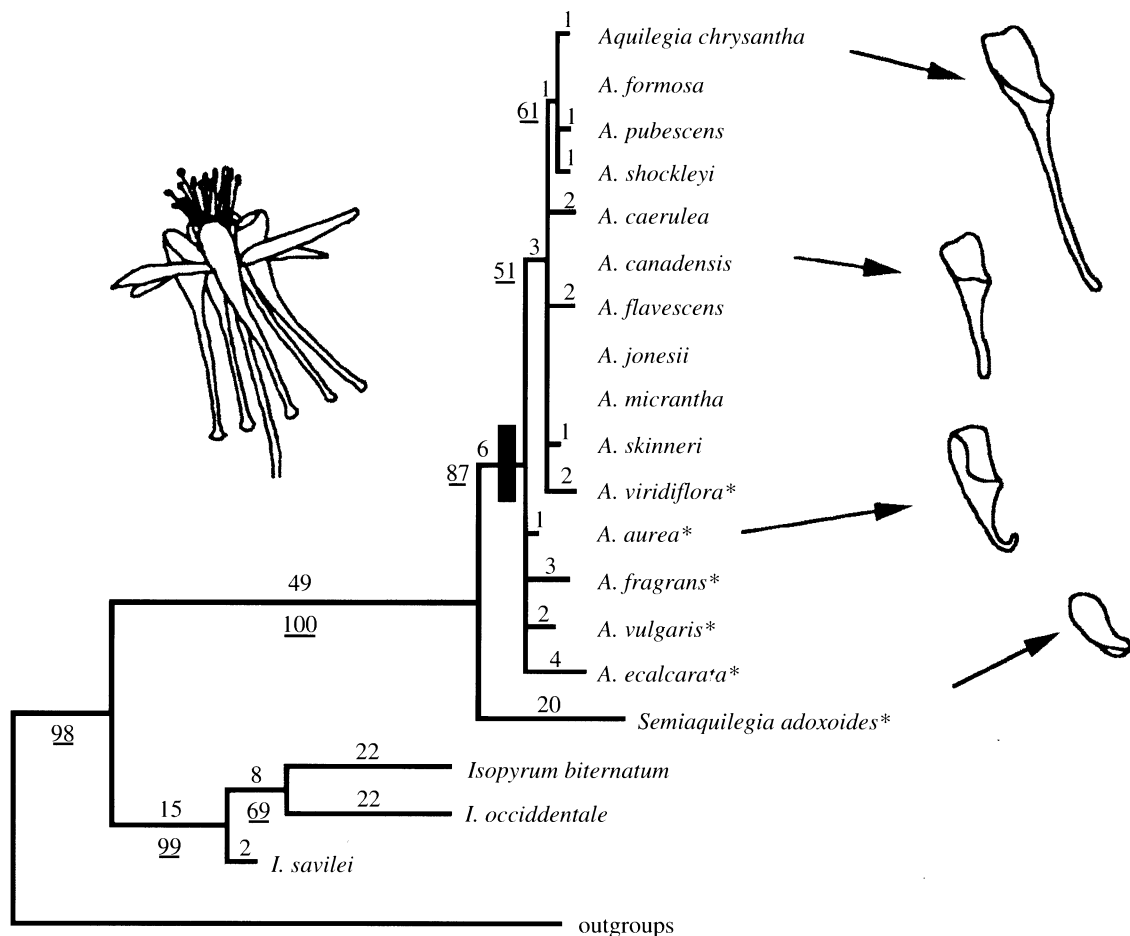


Figure 1. Phylogram of the genus *Aquilegia* and close relatives truncated to only show branches that had bootstrap values over 50%. Old World *Aquilegia* species are indicated (*). Line drawings of individual petals for four species (indicated by arrows) are shown next to the phylogram to demonstrate some of the diversity in shape (petals are not to scale). Also, a line drawing of an individual columbine flower showing the relative placement of petals is provided to the left of the figure. The box on the phylogram indicates the most parsimonious branch for the evolution of nectar spurs. Inferred branch lengths are indicated above each branch and bootstrap values (100 replicates) are underlined, and below or to the left of branches.

speciation can easily be made. Relatively small differences among species in nectar spur morphology are associated with different pollinators and thus changes in nectar spur morphology can provide a prezygotic isolating mechanism leading to increased divergence. Thus, if allopatric populations diverge with respect to floral spur morphology and pollinator type then even if they come into secondary contact the taxa will be reproductively isolated. Similarly, Barraclough *et al.* (1995) recently reported the correlation between sexual dichromatism and species diversity in passerine birds. Because it is thought that sexual dichromatism has evolved through sexual selection, the rate of reproductive divergence and isolation between populations may increase and thus species diversification (Barraclough *et al.* 1995).

A causal link between nectar spurs and increased species diversity in *Aquilegia* is further supported by the apparent close temporal association between the evolution of nectar spurs and the diversification of the columbines (figure 1). Apparently, some event happened along the branch leading to the columbines that did not occur in the *Semiaquilegia* lineage. Because

this branch is short, the number of possible synapomorphies that could be responsible for the columbine diversification is small and the likelihood that the evolution of nectar spurs was the key innovation is increased. The temporal link between the evolution of nectar spurs and increased diversification may be even greater if *Aquilegia ecalcarata* is basal in the columbine clade. However, there is insufficient nucleotide variation to determine the position of *A. ecalcarata* relative to the rest of the columbines (figure 1). Interestingly, crossing studies between *A. ecalcarata* and several spurred species of *Aquilegia* suggest that the presence of nectar spurs may involve a single or very few genes (Prazmo 1965). Thus, nectar spurs may have evolved very quickly within this genus.

Whereas the above data support a key innovation hypothesis for nectar spurs in *Aquilegia*, it is possible that some alternative character led to the columbine diversification. However, we know of no other character that is correlated with the evolution of nectar spurs across the multiple groups we have examined (see table 1) and therefore it is unlikely that a separate synapomorphy is responsible for the diversification of

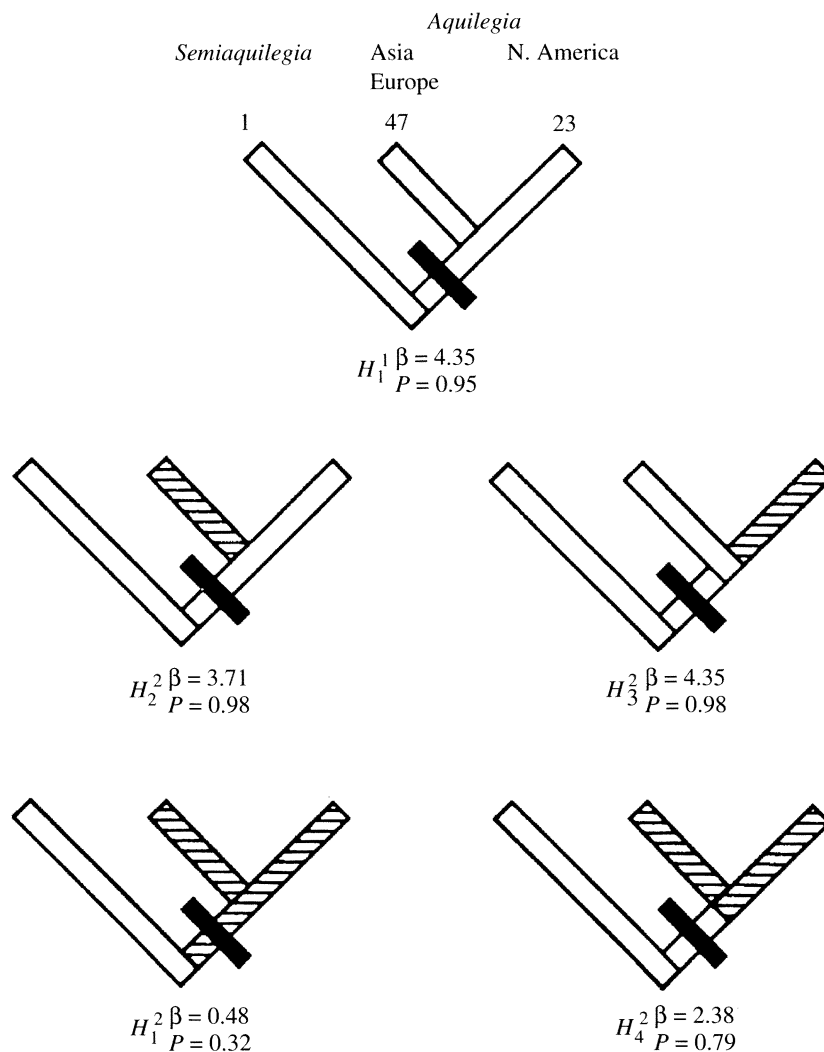


Figure 2. Five models of change in diversification rate (indicated by different shading of branches) used to test if a synapomorphy can be excluded as a key innovation (Sanderson & Donoghue 1994). These models were used to evaluate the evolution of nectar spurs (black bar) as a key innovation in *Aquilegia*. The log-likelihood ratios (β) and corresponding probability values (P) are given for each model using the species diversity for *Semiaquilegia* (one sp.) and the Asian/European and N. American clades of *Aquilegia* (47 and 23 spp., respectively). One Old World species, *A. viridiflora*, groups with all of the New World species (figure 1). Thus, some Old World species that were not sampled in this analysis may group with the New World clade. This would make the species diversity of the two clades more similar and strengthen the findings reported here. The top three models are inconsistent with a key innovation hypothesis. The two two-parameter models on the bottom row, H_1^2 and H_3^2 , are the simplest models consistent with a proposed key innovation (Sanderson & Donoghue 1994).

Table 1. *Species diversity of taxa that have independently evolved floral nectar spurs and their sister groups*

taxa with nectar spurs	no. species	sister taxa	no. species	P	references
<i>Aquilegia</i>	70	<i>Semiaquilegia</i>	1	0.014	this study
		<i>Nigella</i>	14	0.039	(Hoot 1991, 1995)
<i>Delphinium, Aconitum</i>	350	or			
		<i>Nigella, Actaea, Cimicifuga</i>	37	0.095	(Johansson & Jansen 1993)
Fumariaceae	450	<i>Hypericum</i>	15	0.032	†
Tropaeolaceae	88	Akaniaceae,	2	0.022	(Chase <i>et al.</i> 1993;
		Bretschneideraceae			Rodman <i>et al.</i> 1993)
Lentibulariaceae	245	Byblidaceae	2	0.008	(Olmstead <i>et al.</i> 1993;
					Bremer <i>et al.</i> , 1994)
<i>Pelargonium</i>	280	<i>Geranium, Erodium,</i>	399	0.588	(Price & Palmer 1993)
		<i>Monsonia, Sarcocaulon</i>			

† S. Hoot personal comm. In an analysis of *rbcL* sequences from members of the Papaverales, *Hypericum* was found to be the sister group of the Fumariaceae.

these clades. These data are particularly striking as four or five of the six groups with nectar spurs have significantly more species than their sister group (table 1). Additional groups of Angiosperms have independently evolved nectar spurs, including orchids, violets, and *Impatiens*, and it will be interesting to determine if these groups also have increased diversification rates. These determinations await analyses that identify sister taxa.

There are several reasons to suspect *a priori* that some groups with a key innovation may not show significant increases in diversification. For instance, the sister group could evolve a separate innovation and obscure increases in diversification in the lineage of interest. Additionally, a key innovation may be context dependent such that only under certain ecological opportunities will the innovation result in increased diversification. This argument has been made for the evolution of the cichlid jaw apparatus (Liem 1973). This apparatus has been proposed as a key innovation that allowed rapid diversification when cichlids colonized the recently formed, and species depauperate, African rift lakes (Liem 1973). Thus, it is especially remarkable that there is a highly significant association of increased species diversity with the evolution of nectar spurs across many groups.

The single clear exception to the pattern of increased species diversity with the evolution of floral nectar spurs reported here, *Pelargonium* (table 1), has unusual nectar spurs that are 'hidden' (Endress 1994); the spur is fused with the pedicel of the flower. This fusion of floral parts could developmentally constrain the ease in modifying spur shape by requiring two different floral parts to coordinately change. Therefore such a developmental constraint could decrease the ease of diversification and account for the lack of differential diversification found in *Pelargonium*. This hypothesis could be tested because other groups have independently evolved hidden nectar spurs including *Bauhinia*, *Epidendrum*, and *Dactyladenia* (Endress 1994). Thus by identifying sister groups for these taxa different diversification patterns between taxa that have evolved conspicuous nectar spurs and those with 'hidden' nectar spurs could be tested.

The combination of cladistic and statistical analyses used here, along with knowledge of the current effect of a trait on the biology of organisms, provides a strong inference framework to test key innovation hypotheses. As such, we have strong support for the evolution of nectar spurs as an example of a key innovation. Future investigations of traits that affect processes thought to be important in speciation and/or extinction of species may determine whether key innovations have been a common feature of macroevolutionary change.

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