

# Inquiry Figure 30.14\*: Can Flower Shape Influence Speciation Rate?

## Introduction—The Article and Phenomenon Under Study

What evolutionary processes contribute to species diversity? In order for a new species to evolve, there must be genetic divergence and reproductive isolation. Based on current ecological observations and on correlations in the fossil record, we assume the great diversity of flowering plant species is related to animal diversity and to plant-pollinator relationships. The investigator in this paper took a unique approach in testing the hypothesis that flower shape can influence the rate at which new species form. The study compared sister groups of angiosperm families to see the relationship between traits that promote reproductive isolation and increased species diversification. This study, from the following paper, is the subject of Inquiry Figure 30.14 from Campbell/Reece *Biology*, Eighth Edition:

R. D. Sargent, Floral symmetry affects speciation rates in angiosperms, *Proceedings of the Royal Society B: Biological Sciences*, 271:603–608 (2004).

Read the complete article beginning on the next page and then answer the questions following the article.

\* This Inquiry Figure appeared in Campbell/Reece *Biology*, Eighth Edition, as Inquiry Figure 30.14.

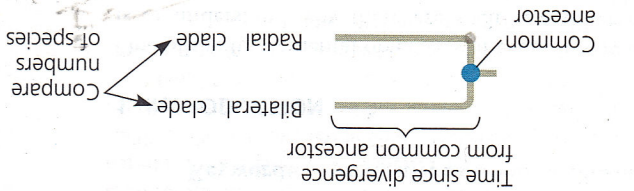
### ▲ Figure 30.14 Inquiry

#### Can flower shape influence speciation rate?

##### EXPERIMENT

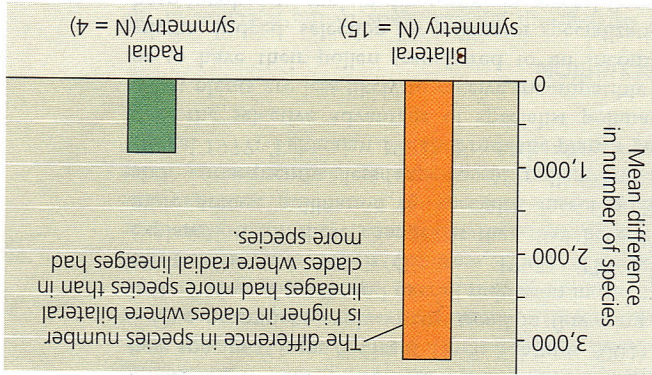
The shapes of bilaterally symmetrical flowers can force pollinators seeking nectar to enter the flower from certain directions only. This facilitates the placement of pollen on particular parts of the pollinator's body, which in turn can increase how effectively pollen is transferred between flowers of the same species. Such specificity in plant-pollinator relationships tends to decrease gene flow between diverging plant populations and hence could lead to increased rates of plant speciation. Risa Sargent, then at the University of British Columbia, tested this hypothesis by determining whether angiosperm families with bilaterally symmetrical flowers contain more species than do closely related families with radially symmetrical flowers, with this approach:

- 1 Identify as many cases as possible in which a clade of one or more families with bilaterally symmetrical flowers is sister to a clade whose members have radially symmetrical flowers. By definition, such sister clades share an immediate common ancestor and hence had the same amount of time to form new species.
- 2 Determine the number of species in each pair of sister clades. The hypothesis would be supported if bilaterally symmetrical clades consistently have more species than their radially symmetrical sister clades.



##### RESULTS

Sargent identified 19 pairs of sister clades that differed in flower shape. In 15 of these pairs, clades with bilateral symmetry had more species than did clades with radial symmetry.



##### CONCLUSION

Sargent's results supported the hypothesis that flower shape affects the rate at which new species form, with bilateral symmetry promoting increased rates of speciation.

##### SOURCE

R. D. Sargent, Floral symmetry affects speciation rates in angiosperms, *Proceedings of the Royal Society B: Biological Sciences* 271:603–608 (2004).

##### Inquiry Action

Read and analyze the original paper in *Inquiry in Action: Interpreting Scientific Papers*.

##### WHAT IF?

Does this study show that flower shape is correlated to the rate at which new species form, or that flower shape is responsible for this rate? Explain.

# Floral symmetry affects speciation rates in angiosperms

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Despite much recent activity in the field of pollination biology, the extent to which animal pollinators drive the formation of new angiosperm species remains unresolved. One problem has been identifying floral adaptations that promote reproductive isolation. The evolution of a bilaterally symmetrical corolla restricts the direction of approach and movement of pollinators on and between flowers. Restricting pollinators to approaching a flower from a single direction facilitates specific placement of pollen on the pollinator. When coupled with pollinator constancy, precise pollen placement can increase the probability that pollen grains reach a compatible stigma. This has the potential to generate reproductive isolation between species, because mutations that cause changes in the placement of pollen on the pollinator may decrease gene flow between incipient species. I predict that animal-pollinated lineages that possess bilaterally symmetrical flowers should have higher speciation rates than lineages possessing radially symmetrical flowers. Using sister-group comparisons I demonstrate that bilaterally symmetric lineages tend to be more species rich than their radially symmetrical sister lineages. This study supports an important role for pollinator-mediated speciation and demonstrates that floral morphology plays a key role in angiosperm speciation.

**Keywords:** reproductive isolation; pollination; sister group comparison; zygomorphy

## 1. INTRODUCTION

One of the fundamental objectives of evolutionary biology is to understand why there are such vast differences in speciation rates across taxonomic lineages (Futuyma 1998). The biological species concept emphasizes reproductive isolation as the key factor in speciation. Consequently, traits that promote reproductive isolation among adjacent populations are considered key to the origin of new species (Grant & Grant 1965; Schluter 2001).

One prominent evolutionary trend in flowering plants is the fusion of petals and overall reduction in the number of stamens and carpels (Endress 1997). The adaptive explanation for these changes is that they have allowed more precise pollination by specialist insect pollinators and, consequently, less expense of pollen and nectar (Regal 1977; Takhtajan 1991). From the plant's perspective, the selective advantage of specialist pollination is clear; plants are less likely to receive incompatible pollen or to have their pollen transferred to an incompatible stigma. Indeed, selection for pollinator specialization has been invoked to explain divergence in several floral traits including: animal pollination, nectar guides, nectar spurs, bilateral symmetry and secondary pollen presentation (Bawa 1995; Waser 2001). Grant (1949) suggested that in the angiosperms, floral morphology has diverged more rapidly than vegetative characteristics, explaining its widespread preference as a basis for taxonomic classification. Many authors suggest that this divergence has been driven largely by selection via pollinators (Grant 1949, 1994; Stebbins 1970; Faegri & van der Pijl 1979; however, see Waser 1998, 2001). Accordingly, the occurrence of animal pollination has been invoked to explain differences in diversification rates across angiosperm lineages (Eriksson & Bremer 1992; Dodd *et al.* 1999).

The importance of pollinator-mediated selection in angiosperms is well supported by theory (Kieffer *et al.* 1984) and experimental data (Galen 1996). In the genus *Mimulus*, evidence suggests that discrimination by pollinators (bees and hummingbirds) is responsible for reproductive isolation between two sympatric species (Schemske & Bradshaw 1999). In the genus *Aquilegia*, differences in the form of nectar spurs are correlated with differences in pollinators that visit a flower; the size and placement of the spurs affect reproductive isolation by reducing visitation by some pollinators and increasing visitation by others (Hodges & Arnold 1994). The presence of spurs has also been shown to correlate with the degree of diversification in other clades, supporting the hypothesis that they play a general role in reproductive isolation (Hodges & Arnold 1995).

Floral symmetry was one of the earliest traits used to relate morphology to function in the pollination of angiosperms (Neal *et al.* 1998). There are two main forms of symmetry described in the angiosperms: bilateral symmetry (zygomorphy) and radial symmetry (actinomorphy). Actinomorphy is considered to be the ancestral form (Takhtajan 1969) with zygomorphy having originated several times independently (Takhtajan 1991; Donoghue *et al.* 1998). Several theories have been proposed for the adaptive significance of zygomorphy (reviewed by Neal *et al.* 1998). The pollen position hypothesis posits that in zygomorphic flowers, pollinators are restricted in the directionality of approach and movement within and between flowers (Leppik 1972; Ostler & Harper 1978; Cronk & Moller 1997). By contrast, actinomorphic flowers can be approached from any direction and are not able to restrict pollinator movement within the flower. Hence, in zygomorphic flowers the specificity of pollen placement is greatly improved. Once precise placement of pollen on the pollinator is achieved, reproductive isolation is possible.

Wherever a trait change has occurred convergently in several lineages there is an opportunity to compare the resulting differences in diversity between the lineage and its sister lineage (reviewed by Barraclough *et al.* 1998). Given sufficient comparisons one can test the hypothesis that the evolution of the trait has had a consistent, replicable effect on diversification. Several studies have examined the impact of different traits on diversification rates in angiosperms (e.g. Farrell *et al.* 1991; Hodges & Arnold 1995; Dodd *et al.* 1999; Heilbut 2000; Barraclough & Savolainen 2001; Verdu 2002) and in other taxonomic groups (e.g. Barraclough *et al.* 1995; Owens *et al.* 1999; Arnquist *et al.* 2000). However, the relationship between floral symmetry and speciation remains untested (Waser 1998). I examine whether zygomorphy has the effect of increasing species richness in the angiosperm lineages where it occurs.

## 2. MATERIAL AND METHODS

### (a) Data collection

I tested the null hypothesis that species numbers in zygomorphic clades were lower than, or equal to, the numbers in their actinomorphic sister clades. I considered symmetry only at the level of the corolla, ignoring the symmetry of the pistil and stamens. Although it is possible to have an actinomorphic corolla and zygomorphic gynoecium or androecium (e.g. *Hibiscus*), or vice versa (Neal *et al.* 1998), I limited the study to corolla morphology because it is the level of symmetry most likely to affect the pollination process (Stebbins 1974). Families in which corolla morphology was defined as zygomorphic were identified using Judd *et al.* (2002). If the information in that source was inadequate, I referred to Watson & Dallwitz (1992) or Mabberley (1997). Families described as having radially symmetrical, polysymmetric or regular corolla morphology were considered actinomorphic; those described as having bilaterally symmetrical, monosymmetric or bilabiate corolla morphology were considered zygomorphic. Only animal-pollinated families were considered.

### (b) Sister-group comparison

Once I had exhausted the listed family descriptions I identified the phylogenetic relationships between these families using the angiosperm phylogeny created by Soltis *et al.* (2000). This is the most complete angiosperm tree currently available; it includes 75% of angiosperm families (Barraclough & Savolainen 2001). Sister-group analyses assume the inclusion of all extant species derived from each branch; hence the interpretation of the results assumes that there is no substantial bias towards actinomorphy in missing families. All the families I had identified as having primarily zygomorphic flowers were found on this tree. Upon identifying a zygomorphic clade I used the Soltis *et al.* (2000) tree to identify the actinomorphic sister clade. This process revealed that several of the zygomorphic families were in fact part of the same lineage. Eventually, 40 zygomorphic families yielded 19 sister group comparisons (figure 1). Once the appropriate sister groups had been identified I used Mabberley (1997) to determine the number of species in each family. In cases where Mabberley (1997) disagreed with the taxonomic divisions in the Soltis *et al.* (2000) phylogeny, I used other sources (Watson & Dallwitz 1992 or Judd *et al.* 2002) to determine the number of species in the lineage. Occasionally, the zygomorphic families (e.g. Fabaceae) contained some actinomorphic members. Using methodology described in Farrell *et al.* (1991) and Heilbut (2000), I reported the number

of species for the sister group as the total minus the number of actinomorphic species (figure 1). Similarly, in one case (Zingiberales) a group of taxa having wind-pollinated flowers (Poales) was removed from the zygomorphic sister-group total for the comparison. This procedure was conservative and could only bias the results against rejecting the null hypothesis. The reciprocal procedure (subtracting zygomorphic species from actinomorphic clades) was not performed; this also ensured that the test was conservative. While most sister groups represented independent comparisons, I included one sister pair (Polygalaceae–Surtanaceae) that fell within the zygomorphic sister lineage of another pair (Fabaceae and its sister group). I controlled for any possible bias that this approach could have caused by subtracting the species from the Polygalaceae–Surtanaceae comparison from the more inclusive sister group (leaving only the Fabaceae), thus assuming that one large group was not providing the basis for more than one positive comparison. However, removing this additional pair does not change the significance of the results reported below.

### (c) Statistical tests

To determine whether there was a significant effect of the evolution of zygomorphy on the diversification rate of a lineage, I subtracted the number of species in the zygomorphic lineage from the number of species in the actinomorphic sister lineage. I tested whether there was a detectable trend in the direction of the differences using a one-tailed sign test and by testing whether the mean difference in species number between sister groups differed from zero using the non-parametric Wilcoxon signed-rank test. Results are reported as means  $\pm$  one standard error.

## 3. RESULTS

In 15 out of 19 sister-group comparisons the lineage with zygomorphic flowers was more diverse than its sister group (table 1; figure 1; figure 1;  $p = 0.0096$ , one-tailed sign test). Furthermore, the mean difference in species number between the sister groups was significantly greater than zero (table 1;  $n = 19$ ,  $p = 0.003$ , Wilcoxon signed-rank test). The mean negative difference (actinomorphic clade contains more species) was  $847.75 \pm 758.17$  and the mean positive difference (zygomorphic clade contains more species) was  $3318.53 \pm 1688.07$ .

## 4. DISCUSSION

The sister-group analysis leads to the rejection of the null hypothesis in favour of the alternative hypothesis that bilaterally symmetrical (zygomorphic) clades are more species-rich than their radially symmetrical (actinomorphic) sister clades. This conclusion is consistent with field studies reporting an association between zygomorphy and species richness. In their study of 25 flowering plant communities, Ooster & Harper (1978) found that zygomorphy was correlated with increased plant diversity. Their explanation for this result is that in species-rich communities, zygomorphy should be favoured because it promotes increased fidelity between flowers of a given species and their pollinators. It has been proposed that the evolution of zygomorphy will lead to increased speciation rates because it affects the precision of pollen transfer and hence the probability of

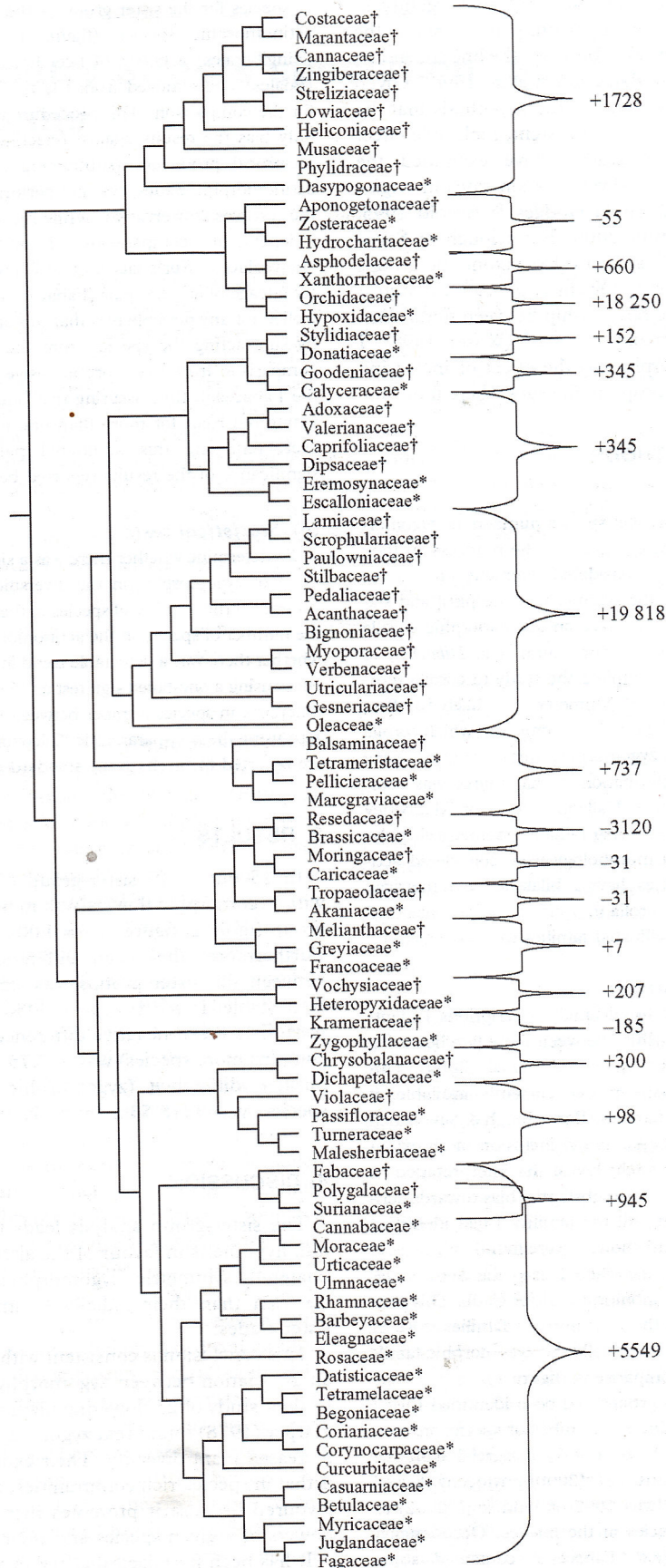


Figure 1. Phylogeny of zygomorphic angiosperm families and their sister taxa adapted from Soltis *et al.* (2000). Braces indicate the 19 sister-group comparisons. The number opposite each brace indicates the difference in species number between the two sister groups (zygomorphic species minus actinomorphic species); a dagger indicates a zygomorphic family; an asterisk indicates an actinomorphic family.

Table 1. Sister group comparisons for zygomorphic families. (Only animal-pollinated families were used. The numbers given have been corrected against this bias by removing actinomorphic members of the zygomorphic clade from the total (see text for details). The final column indicates the outcome of the sister-group comparison; + indicates that the zygomorphic clade had more species; - indicates that the actinomorphic clade had more species.)

zygomorphic family	number	sister group	number	+/-
Acanthaceae (3450) + Bignoniaceae (750) + Gesneriaceae (2900)	20433	Oleaceae	615	+
+ Lamiales (6700) + Myoporaceae (235) + Paulowniaceae (6)				
+ Pedaliaceae (85) + Scrophulariaceae (5100) + Stilbaceae (12)				
+ Verbenaceae (950) + Urticariaceae (245)				
Adoxaceae (5) + Caprifoliaceae (420) + Dipsacaceae (290)	1015	Eremosynaceae (150) + Escalloniaceae (1)	151	+
+ Valerianaceae (300)				
Aponogetonaceae				
Asphodelaceae	43	Hydrocharitaceae <sup>b</sup> (80) + Zosteraceae (18)	98	-
Balsaminaceae	750	Xanthorrhoeaceae	90	+
Cannaceae <sup>a</sup> (8) + Costaceae (1100) + Heliconiaceae (80)	850	Marcgraviaceae (108) + Pelliceraceae (1) + Tetrameristaceae (4)	113	+
+ Lowiaceae (7) + Marantaceae <sup>a</sup> (535) + Mimosaceae (200)	1823	Dasyopogonaceae	95	+
+ Phyllidaceae (6) + Strelitziaceae (7) + Xyridaceae <sup>b</sup> (300)				
+ Zingiberaceae (1100)				
- Bromeliaceae (1520)				
Chrysobalanaceae	460	Dichapetalaceae	160	+
Goodeniaceae <sup>a</sup>	400	Calyceraceae <sup>a</sup>	55	+
Krameriaceae	15	Zygophyllaceae	200	-
Fabaceae <sup>b</sup> (15315) - Polygalaceae <sup>b</sup> (950) - Surianaceae (5)	14360	Barbeyaceae (1) + Begoniaceae (900) + Berulaceae (110)	8811	+
		+ Cannabaceae (4) + Casuarinaceae (95) + Coriariaceae (5)		
		+ Corynocarpaceae (4) + Cucurbitaceae (775) + Datisicaceae (4)		
		+ Elaeagnaceae (45) + Fagaceae (700) + Juglandaceae (59)		
		+ Moraceae (1100) + Myricaceae (55) + Rhamnaceae (900)		
		+ Rosaceae (2825) + Tetramelaceae (4) + Ulmaceae (175)		
		+ Urticaceae (1050)		
Melasthaceae	12	Pranocaceae (2) + Greyiaceae (3)	5	+
Moringaceae	12	Caricaceae <sup>a</sup>	43	-
Orchidaceae	18500	Hypoxidaceae	220	-
Polygalaceae <sup>b</sup>	950	Surianaceae	5	+
Resedaceae	80	Brassicaceae	3200	-
Syridiaceae	154	Donatiaceae	2	+
Tropaeolaceae	89	Akanaceae	1	+
Violaceae	800	Malsherbaceae (27) + Passifloraceae (575) + Turneraceae (100)	702	+
Vochysiaceae <sup>b</sup>	210	Heteropyxidaceae	3	+
total				15+/4-

<sup>a</sup> Entire family displays secondary pollen presentation.

<sup>b</sup> Some members display secondary pollen presentation.

reproductive isolation arising among slight variants (Neal *et al.* 1998). If this were true, we would expect zygomorphy to be correlated with either specialist pollinators or the placement of pollen on specific parts of a pollinator's body. Additionally, I predict that other traits that require precise pollen transfer in order to have a selective advantage, such as lower pollen-ovule ratios, will be correlated with zygomorphy.

Indeed, an association between zygomorphy and pollination by specialist bees has been reported in several angiosperm taxa (Donoghue *et al.* 1998; Goldblatt *et al.* 2000). Specialist pollinators clearly have the potential to increase diversification rates. Bumble-bee pollinators may prefer zygomorphic to actinomorphic forms (Neal *et al.* 1998). In addition, bees may be inefficient pollinators of actinomorphic flowers (Cronk & Moller 1997). Moreover, reversals to actinomorphy may accompany a switch from specialist to generalist pollinators (Cronk & Moller 1997; Donoghue *et al.* 1998). There is also evidence suggesting that in some species with zygomorphic flowers, pollen placement is so precise that the same pollinator can visit multiple species and preserve reproductive isolation because the pollen is placed on different parts of the pollinator (Brantjes 1982, 1985). While further exploration is required, a correlation between zygomorphy and specialist pollinators further supports the hypothesis that higher species richness in zygomorphic lineages results from pollinator-mediated speciation.

If zygomorphy promotes reproductive isolation via improved placement of pollen, we would expect that the pollen-ovule ratio in species with zygomorphic flowers would evolve to be lower than in species with actinomorphic flowers. It has been demonstrated that the amount of pollen produced by a species (measured as the pollen-ovule ratio) is negatively correlated with the likelihood that the plant's pollen grains will reach a compatible stigma. For example, animal-pollinated plants have lower pollen-ovule ratios than wind-pollinated plants (Sharma *et al.* 1992), and plants that are obligately selfing (autogamous) have lower pollen-ovule ratios than those that obligately outcross (Cruden 1977). There is indeed some evidence that species with zygomorphic flowers have lower pollen-ovule ratios. For example, in the Orchidaceae, pollen is packaged into units known as pollinaria, which results in a pollen-ovule ratio that is several orders of magnitude smaller than in plants that lack these structures. The evolution of pollinaria has been directly attributed to the improved specificity accompanying the evolution of zygomorphy (Johnson & Edwards 2000). The pollinaria have been championed as a key innovation that allowed the rapid diversification of the orchid clade (Johnson & Edwards 2000). However, without a preceding adaptation to ensure highly specific pollination, pollinaria would be disadvantageous. In the Asterales, lineages that develop zygomorphy have often undergone a subsequent decrease in anther number (Endress 1998). While there are other possible explanations for this trend, it is an intriguing observation that deserves further exploration.

A potential problem with any sister-group analysis is that the examined trait (in this case zygomorphy) could be correlated with a different trait that drives diversification rather than be the actual cause of the diversification. This is an intrinsic problem with all correlative

studies. The presence of secondary pollen presentation, i.e. the presentation of pollen on floral structures other than the anther sacs (Yeo 1993), is also correlated with low pollen-ovule ratios (Cruden 2000), reportedly due to its ability to facilitate highly specific placement of pollen grains (Howell *et al.* 1993). Because of its purported role in improving pollination efficiency, secondary pollen presentation is another candidate trait that may play a role in angiosperm speciation. In addition, many families that display secondary pollen presentation also have zygomorphic flowers. Therefore, I repeated the sister-group comparison, excluding species or families that displayed secondary pollen presentation (table 1) to test whether secondary pollen presentation could have driven the association between zygomorphy and species richness. When these species are removed, only one comparison (Fabaceae and its sister lineage) is reversed, and the sign test remains significant ( $p = 0.0155$ ). Because secondary pollen presentation is not strongly correlated with zygomorphy (table 1), it is unlikely to be driving the observed patterns of diversification. Secondary pollen presentation may also work in conjunction with zygomorphy in some families to ensure precise pollen placement (Yeo 1993).

A major weakness of a sister-group analysis is that it cannot distinguish whether differences between sister lineages in species richness are caused by more speciation events in one lineage or by more extinction events in the other. In the present case, however, there is no reason to expect that actinomorphy would increase extinction rates. Rather, actinomorphy may lead to lower extinction rates because of its association with generalist pollinators (Bond 1994; Johnson & Steiner 2000).

Finally, it is possible that zygomorphy affects speciation rates in a manner unrelated to its ability to promote reproductive isolation. For example, if reproductive assurance is greater in zygomorphic clades, they might be less susceptible to extinction. By contrast, zygomorphy may affect pollinator constancy such that competing species are more prone to extinction. The analysis presented here does not allow one to distinguish between these hypotheses.

In conclusion, I have argued that the correlation between zygomorphy and increased species richness in angiosperms is caused by the ability of this trait to promote reproductive isolation through improved precision of pollen placement and the tendency for specialist pollinators to be attracted to zygomorphic flowers. This study is distinctive in that it investigates a trait long suspected to be important in reproductive isolation and confirms a hypothesis central to evolutionary biology: traits that promote reproductive isolation are correlated with increased diversification rates.

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## REFERENCES

*Guiding Questions for Reading This Article*

**A. About the Article**

1. Name the journal and the year in which this study was published.
2. The author is affiliated with what university? In what country?
3. Funding for the project was provided through what agency?
4. Specialized vocabulary: Write a brief definition of each term.

*Described in the article text:*

actinomorphy

zygomorphy

*Not defined in the article:*

androecium

clade

corolla

gynoecium

ovule

phylogeny

**B. About the Study**

5. What is the adaptive explanation for the prominent evolutionary trend toward flowers with fused petals and reduced number of stamens and carpels?

6. Give two examples of specialized floral traits, and state the advantage of having such traits, from the plant's perspective.

7. State the genus names of two flowering plants for which experimental results suggest that reproductive isolation between two species is related to visitation by different pollinators.

8. Explain the proposed adaptive advantage of zygomorphy for improving specific pollen placement.

9. What was the source of the angiosperm phylogeny used for the sister-group comparison in this study, and why was that source chosen?

10. What is a null hypothesis, and what is the specific null hypothesis tested in this study?

11. In the process of science, proper hypothesis testing requires an experimental design with replication—that is, repeated independent tests of the same hypothesis with different data sets. How did this investigator design a study with replication?

12. The brackets at the left side of Figure 1 indicate phylogenetic groupings among zygomorphic angiosperm families, and the braces at the right of the figure indicate sister groups. (a) How many sister groups were compared in this study? (b) Look down the list and find the brace that includes the violet family, Violaceae. List the plant family names for that brace. (c) What do the brackets for the families in this brace indicate about the phylogenetic

Violaceae brace families is zygomorphic and which actinomorphic? (See figure legends for symbols.) (e) For the families in this brace, what is the number of zygomorphic species minus the number of actinomorphic species? (f) In this brace, which group has more species—the zygomorphic or the actinomorphic groups?

13. Do the results of the zygomorphic-actinomorphic comparison lead us to *reject* the null hypothesis or *fail to reject* the null hypothesis? Explain your answer.

### C. General Conclusions and Extensions of the Work

14. How does the conclusion from this study compare to the results of field studies of zygomorphy and species richness in specific plant communities?
15. What relationship between zygomorphic floral structure and pollinator behavior has been observed in other research?
16. With any observation, an observed correlation does not always mean causation. (a) What is a potential problem (correlation causation) of the sister-group analysis of zygomorphy? (b) What *other* structural adaptation, besides zygomorphy, did the author suggest could be an alternative cause of the low pollen-ovule ratio that was observed? (c) How did the investigator address this alternative explanation? (d) What was the conclusion after the alternative explanation was eliminated?

17. What is a major limitation or weakness of the sister-group analysis for studying the relationship of zygomorphy and speciation?
18. What is the reasoning for predicting that plant species with zygomorphic flowers would evolve a *lower* ratio of pollen produced to number of ovules produced? Are there any plants that show such a low pollen-ovule ratio?
19. In concluding, the author states that “the correlation between zygomorphy and increased species richness in angiosperms is caused by the ability of this trait to promote reproductive isolation through improved precision of pollen placement and the tendency for specialist pollinators to be attracted to zygomorphic flowers.” Do you think that her study provides proof of (a) that correlation and (b) that causation?
20. Imagine that you were working with this researcher. What could be a possible follow-up test that extends this work?