Evolution: Pollen or Pollinators — Which Came First?

A new study provides the first broad timeline of bee diversification. Several ancient bee clades are identified as ghost lineages that have left little fossil evidence of their existence. This timeline suggests that the rise of bees coincided with the largest flowering plant clade, the eudicots.

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Flowering plants and their insect pollinators are often presented as one of the most remarkable examples of co-evolution on earth. Among many insect visitors, bees are one of the most important groups of flowering plant pollinators [1]. They include some 20,000 species that depend almost exclusively on resources produced by flowers such as pollen and nectar [1] (Figure 1). Bees are also very important to humans — approximately one third of our diet from vegetables and fruits are the result of pollinator services provided by bees [1]. Despite their importance, however, the precise timing of their presumed co-diversification has remained elusive. The bee fossil record is relatively poor but indicates that they might have arisen in the mid-Cretaceous, roughly 140–110 million years ago (Mya), which is consistent with fossil origins of flowering plants [2]. However, the extent to which their diversification coincided with that of flowering plants remains unclear.

In the absence of a rich fossil record, dated molecular phylogenies provide an essential tool to reconstruct the tempo and mode of diversification of the history of life on earth. The era of molecular phylogenetics and the integration of newly identified fossils have helped untangle the origin of flowering plants and to identify the timing of diversification of its major clades [3–5]. The same cannot be said for our understanding of the bees because a comprehensive dated phylogeny of its major lineages has been lacking. A recent study by Cardinal and Danforth [6] helps to untangle patterns of bee diversification, and the extent to which their timing coincided with that of their floral host plants.

Cardinal and Danforth sampled seven genes broadly across all currently recognized bee families, subfamilies, and most tribes. They then dated their DNA phylogeny using 14 fossil age constraints selected from the ~200 described bee fossils [7]. Their findings demonstrate that most major bee lineages (e.g., families) originated during the Cretaceous (132–113 Mya), which indicates that these lineages are much older than their fossil record [7]. One explanation for this discrepancy is the apparent preservational bias of bee fossils [7]. Most of these fossils represent lineages of resin-collecting bees from the northern hemisphere, which tend to be well preserved in amber. However, resin-collecting bees are phylogenetically concentrated in only two of the seven bee families (Apidae and Megachilidae), which makes determining the origins of most other bee clades difficult. These molecular divergence time estimates indicate that numerous bee clades exist as ghost lineages that lack fossil representation for much of their evolutionary history. One should be cautious about molecular divergence estimates that appear out of sync with the fossil record [8]; however, some lines of evidence support their older age estimates. For instance, their dated bee phylogeny indicates an older origin for sweat bees (Halictidae; 75–96 Mya) than the oldest known fossils for this group (53 Mya) [7]. Evidence from fossilized nests previously attributed to sweat bees from the Late Cretaceous [9], however, corroborate these older ages. On the other hand, the inferred Miocene (20 Mya) origin of the leaf-cutter bees (Megachilini) contradicts much older (Mid-Eocene; ~45 Mya) fossilized leaf damage by these bees. Here, Cardinal and Danforth identify insufficient taxon sampling in their study as a likely reason for these overly young age estimates.

This new timeline of bee diversification is an important leap forward and an essential tool for comparative evolutionary biology. Their results are particularly exciting for one main reason: crown group bees originated ~125 Mya, which roughly coincides with the origin of eudicot angiosperms. Eudicots are defined by distinctive tricolpate pollen (i.e., pollen with three pores), which is easily recognized in the fossil record. The eudicots comprise 70% (165,000 species) of flowering plants and include most of the broad-leaved trees and

Figure 1. Bee and eudicot diversity.

Images above showing representatives of major bee clades, including sweat bees (Halictidae; courtesy of S. McCann), bumble bees (Apidae; courtesy of C. Quintin), and leafcutter bees (Megachillidae; courtesy of I. Marsman). Images below showing major eudicot clades, including roses (Rosaceae; courtesy of M. Green), legumes (Fabaceae; courtesy of A. Dang), and sunflowers (Asteraceae; courtesy of M. Brenn). All images are used under a Creative Commons license.

shrubs that populate our forests, plus ecologically and economically important clades like legumes, roses, and sunflowers. Surprisingly, it is not the origin of the flower (specifically, the carpel) that coincides with a substantial burst in angiosperm diversification, but it is rather the origin of eudicots at ~125 Mya where a burst in diversification has occurred [4]. The direct cause of this diversification remains unclear, but one intriguing possibility is that eudicot diversification was attributable to their mutualistic interaction with bees [10,11]. This raises the tantalizing possibility that the origin of pentamerous flowers in eudicots [3] and their specialization on bees may have spurred co-diversification in both groups.

But did crown group bees and eudicots co-diversify during this dramatic window of angiosperm evolution? And if bees did radiate during this time period, which came first, the bees or eudicot pollen? We do not know the answer to the first question because the authors did not specifically test for a burst in diversification when crown group bees originated 125 Mya. The second question to determine the precise timing of this co-diversification is much more difficult to answer. A recent attempt by Ramírez and colleagues [12] examined rates of co-diversification between Neotropical orchid bees (Euglossini) and perfume-producing orchids, which represents a remarkable and highly specialized plant–pollinator mutualism. In this system, male bees gather fragrances from orchids and use them to attract females for mating. Their analyses, incorporating dated phylogenies for both the bees and the plants, demonstrated that a shift in orchid diversification (30–15 Mya) likely occurred subsequent to a recent diversification (40–27 Mya) in orchid bees. Thus, the timing of this interaction appears to have been decoupled: fragrance collection by bees appears to have preceded the evolution of fragrance-producing orchids, suggesting that floral host shifts may have played a role in the origin of this plant–pollinator mutualism.

The orchid-bee–orchid-flower example [12] nicely illustrates that prolific diversification between bee–plant mutualisms may not be perfectly coincident, and importantly, that they are much younger than the origin of eudicots and crown group bees (Figure 2). These more recent bursts of speciation are likely true for other highly specialized mutualisms as well. Oil-gathering bees (Centridini) that collect floral oils to construct nests and provide food for their larvae are a nice example [13]. Floral oil production has evolved at least ten times within angiosperms [14]. In one of these clades, the Barbados cherry family (Malpighiaceae), which originated ~75 Mya, there is evidence that their exceptional diversity (1,100 spp.) traces to their specialist association with these oil-gathering bees [15,16]. A preliminary assessment points to other more recent radiations involving this specialized oil-bee–oil-plant mutualism (Figure 2). Perhaps the most striking example are the oil-rewarding *Calceolaria* species that arose very recently (6 Mya), yet whose diversity exceeds 200 species [17]. The precise timing of co-diversification in this, and other such bee–plant mutualisms, however, remains untested. This is largely attributable to the lack of dated phylogenies for each of these mutualistic partners, which is necessary to begin linking speciation patterns with specialization in these systems. The new dated bee phylogeny by Cardinal and Danforth marks an important step in this direction, which hopefully will establish a new sort of mutualistic interaction — one between flowering plant and insect phylogenetic biologists seeking to resolve the prolific rise of flowering plants.

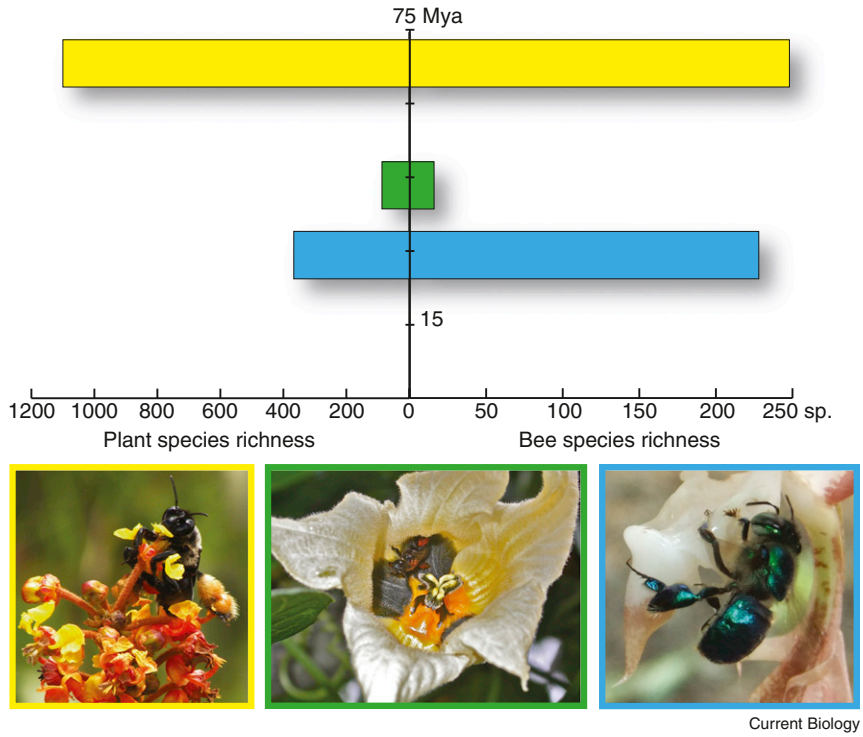

Figure 2. Diversity of specialized plant–pollinator mutualists.

Species diversity and approximate crown group ages for bees and angiosperms involved in specialized pollination systems. Oil-rewarding flowers of Malpighiaceae (yellow; courtesy of S. Cappellari) and Cucurbitaceae (green; courtesy of H. Schaefer) with oil-collecting bees (Centridini and Ctenoplectrini, respectively) [14,18,19]; fragrance-producing Orchidaceae (blue; courtesy of I. Morton, Creative Commons license) associated with orchid bees (Euglossini) [1,12,20].

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Small RNA-Directed Silencing: The Fly Finds Its Inner Fission Yeast?

Several recent studies demonstrate that piRNAs guide Piwi protein to repress transposon transcription in fly ovaries, much as fission yeast use siRNAs to silence repeat sequences. Still mysterious though is how Piwi targets euchromatic transposons for silencing, but not the specialized heterochromatic loci that produce piRNA precursors.

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Fungi, plants, and animals devote considerable resources to thwart transposable elements from increasing their numbers or moving to new genomic locations, particularly in germ cells. In fungi and plants, small interfering RNAs (siRNAs) act via the RNA interference (RNAi) pathway to silence transposons and other types of repetitive DNA. In contrast, animals use PIWI-interacting RNAs (piRNAs), a class of small silencing RNAs distinct from siRNAs, to silence germline transposons and ensure fertility. Like siRNAs and the mRNA-regulating microRNAs (miRNAs), piRNAs direct Argonaute proteins to silence complementary nucleic acid targets. Unlike siRNAs and miRNAs, piRNAs guide a specialized sub-class of Argonautes, the PIWI proteins, which are found exclusively in animals and nearly always in the germline or germline-related cells.

In *Drosophila*, piRNAs bind three different PIWI proteins: *P*-element-*i*nduced *w*impy *t*estes (Piwi), Aubergine (Aub), and Argonaute3 (Ago3). Aub and Ago3 act strictly in the ovary and testis germline, where they silence transposons by destroying their RNA transcripts. In contrast, Piwi resides in the nucleus, where it represses transposons in both germ cells and their supporting somatic cells [1–3]. Now, four papers demonstrate that Piwi silences transposons, at least in part, by repressing their transcription [4–7]. These genome-scale studies support and extend earlier evidence that Piwi directs transcriptional silencing in the nucleus [3,8,9]. By depleting Piwi in the ovarian germline [5,6], ovarian somatic follicle cells [6], or cultured, immortalized ovarian somatic cells (OSCs) [4], or by inserting ectopic piRNA target sites into the fly genome [7], all four studies find that piRNAs guide Piwi to its target loci, where it recruits enzymes that establish repressive heterochromatin (Figure 1A). The papers generally support the view that piRNAs tether Piwi to nascent transcripts: RNA is required for Piwi to co-immunoprecipitate with chromatin [7] and with proteins known to bind nascent RNA [5]. Piwi bound to nascent RNA via its piRNA guide appears to recruit Su(var)3-9 [7], a histone methyltransferase that methylates histone H3 on lysine 9. These 'H3K9me3' marks bind heterochromatin protein 1 (HP1, officially named Su(var)205), generating chromatin that is refractory to transcription, as reflected by reduced occupancy with RNA polymerase II (pol II) [7]. Supporting this view, depletion of Piwi reduces the amount of H3K9me3 [4–6,9] and HP1 [9] and increases the amount of RNA pol II [4,5] and nascent transcripts [4,6,8] at transposon sequences.

These findings call to mind the mechanism by which the RNAi pathway silences repetitive sequences in the fission yeast, *Schizosaccharomyces pombe*. siRNAs bound to *S. pombe* Ago1 guide the 'RITS' complex to nascent transcripts from transposon-like repeats near the centromere, where it recruits proteins that establish repressive