

Cenozoic insect–plant diversification in the tropics

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Evolution has done wonders with mass extinctions. Organic diversity has rebuilt itself at least five times during the history of life on Earth, fashioning novelty from the organic remnants of each catastrophe (1). Modern biodiversity arose from a global extinction event at the end of the Cretaceous period (the “K-T” extinction) that probably was caused by an asteroid or comet impact. This penultimate mass extinction [that before the recent and incipient human-caused extinctions (2)] eliminated 80% of large plant species, 20% of marine animal families, all dinosaurs except ancestors of present-day birds, and probably many specialized insect–plant associations (3). Some of the lineages that pushed through the end-Cretaceous bottleneck have done extremely well during the ensuing 65 million years of often favorable Cenozoic environments. Bony fishes, corals, and several invertebrate groups enjoyed net cladogenesis and speciation that greatly exceeded extinction. On land, groups with a good start before the end-Cretaceous extinction and impressive radiation since include birds, mammals, insects, and angiosperms. Stebbins (4) inspired modern research on recovery from extinction with the metaphor that plant communities are simultaneously “museums,” which preserve ancient lineages, and “cradles,” which foster speciation. The power of science to address these issues has been greatly increased by the advent of molecular phylogenetics, which when complemented by paleontology, biogeography, evolution, and ecology, can give a picture of the tempo of recovery. In this issue of PNAS, McKenna and Farrell (5) address the temporal trajectories of diversification rates and, by inference, the mechanisms of recovery from the end-Cretaceous mass extinction of the specialized and speciose *Cephaloleia* leaf beetles of Middle America.

In a previous article (6) McKenna and Farrell brought together intensive taxonomy and the basic molecular phylogeny of this genus and its relatives. These are ancient beetles that have long specialized upon a single clade of monocots, the Zingiberales. The distinctive feeding damage of *Cephaloleia* ancestors on fossilized ginger leaves dating from late in the Cretaceous and early in the Eocene demonstrated that these radiations of leaf beetles began some 20 mil-

lions of years (Ma) earlier than the first body parts of leaf beetles are known in the fossil record (7).

Diversification Rates

McKenna and Farrell (5) present two main conclusions regarding diversification rates in *Cephaloleia*. First, they infer an across-clade slowdown in rate over time. Second, they localize the most significant shifts in diversification rate to the Eocene, with some additional shifts in the Oligocene in nested clades arising subsequent to one of these shifts. These analyses are simultaneously a tour de force of statistical phylogenetic techniques and a cascading set of dependencies of one inference on another. At the head is a phylogenetic tree of the insects, relying on a now-standard set of molecular sequence markers and the conventional arsenal of tree-building

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techniques. Next is an analysis of divergence times of the nodes in this tree by using penalized likelihood, one of a growing set of so-called “relaxed clock” procedures that attempt to infer clade ages from sequence data without a strict requirement of clocklike molecular rates. This analysis combined calibrations from three insect fossils, used as minimum age constraints within the in-group, and a range of estimated ages (120–145 Ma) of their monocot host plants as maximum age constraints. Finally, based on the resulting “chronogram,” a plot of the number of lineages through time (LTT) was used to examine the tempo of speciation and extinction across the group. The shape of this plot can reveal relative speciation and extinction rates and in some cases detect changes in one or both. However, LTT analyses must also contend with the confounding interactions between speciation rates, generating the observed diversity; extinction rates, which lead to a censoring of the observed diversity and some odd pathologies in the statistical analysis (8); and partial sampling of

the extant species, which also affects the shape of the LTT plot. To detect significant rate shifts, McKenna and Farrell applied a relatively little known test, the relative cladogenesis test (9), that generalizes the earlier and more widely used Slowsinski–Guyer (SG) sister-group test (10). This test looks for unusually species-rich clades in the cohort of clades all originating at one slice of time in the tree and can be more powerful than the SG test in identifying subtle shifts owing to the replication afforded by the cohort beyond the two clades in the SG test.

Naturally, the strength of this chain of inferences depends on each link. There is little reason to question the basic phylogenetic results; they rest on now-standard technology. The diversification rate analyses do, however, all depend critically on the estimated divergence times. In any “reconstructed tree” (i.e., a tree in which any extinction lineages are removed, as in any tree built entirely from sequence data; ref. 11), the lineages-through-time plot will imply that the ages of nodes are pulled toward the root if few terminals are sampled, pulled toward the recent if extinction is rampant, and pulled in either direction depending on whether and where speciation or extinction rates change over time. In a relative cladogenesis test, the number of lineages present at any instant in time is also directly determined by the divergence times of the tree’s nodes.

If molecular clocks were common features of evolution, then given enough data, one could estimate all divergence times to within a level of accuracy determined basically by the error in fossil calibration dates. Absent a clock, the problem is harder, but there has been no shortage of statistical muscle applied to it. Unfortunately, theoretical results (11) indicate, unlike in the clock case, that the accuracy of any given divergence time cannot be improved beyond a certain point with the mere addition of more sequence data; instead, some combination of additional fossils or additional genes whose pattern of lineage-specific rate variation is different is

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necessary. These results are in accord with much empirical work on divergence times that argues for more attention to the number and quality of the fossil calibrations (12). Unfortunately, present assessments of confidence intervals on divergence times are not able to overcome systematic biases or model misspecification (with respect to rate changes), which leaves us able to do little else but explore the robustness of the analyses to perturbation. The present analysis uses several calibration points, and it would be interesting to gauge the effect of each by sequentially removing them.

Host Plant and Beetle Radiation

McKenna and Farrell (5) infer that multiple extant clades of *Cephaloleia* radiated in Paleocene–Eocene (circa 55–44 Ma) times of global warming when their Zingiberales host plants radiated and expanded their geographical ranges to higher latitudes. This evidence for the ancient origin of *Cephaloleia* complements the evidence from the fossil feeding damage (7), providing another important example of today's Neotropical forests as important museums of their biota. They also imply the timing of a more recent group of *Cephaloleia* clades in the Oligocene (circa 32 Ma), which radiates upon *Heliconia* and feeds exclusively upon these plants. This finding dates the radiation of another specialized *Cephaloleia* clade that is restricted to feeding in the immature rolled leaves of Marantaceae and Heliconiaceae as adults and possibly also as larvae (8, 9). It is as if these scores, or perhaps hundreds, of closely related beetle species were a Grand Prix of evolutionary slot cars running for millions of years down courses set by their host plant clades. The denouement of this fascinating evolutionary story is that the Neotropics are also cradles for these beetles. Abun-

dant recent Miocene–Pliocene diversification of *Cephaloleia*, timed with the closing of the oceanic gateway between North and South America, generated a “crown” diversification of *Cephaloleia* coincident with the crown diversification of *Heliconia* hosts. McKenna and Farrell find that the extant sister species of *Cephaloleia* arising in the Eocene (<33.9 Ma) in their analysis are allopatric or parapatric, which suggests that this recent speciation has happened largely by isolation associated with the closing of the Isthmus of Panama.

Niches and Diversification

From an ecological perspective, an extremely interesting aspect of McKenna and Farrell's results (5) is the apparent convergence long ago to the habit of living and feeding within rolled *Heliconia* leaves with *Cephaloleia* of beetles in two related genera, *Alurnus* and *Chelobasis*. The authors suppose that these three genera “have coexisted in immature rolled *Heliconia* leaves for a remarkably long period.” They reasonably infer that the rolled leaves of host taxa are crucial to the definition of ecological niches into which these beetles can diversify. Earlier work (13) with the community ecology of these insects concluded that species of these three genera coexisted within single rolled leaves and in patches of their hosts without contemporary interspecific competition. No evidence was found in this earlier work of niche differentiation (i.e., that the coexisting species in the three beetle genera used their single host leaf, patch, or species differently). Densities were very low relative to the amount of habitat and food for the beetles in rolled leaves, probably because of very high mortality imposed by natural enemies, parasitoids, and predators. Thus, by reducing densities of these beetles, these natural enemies probably played an im-

portant role in defining the number or breadths of the niches that would protect the different species from competitive exclusion for so many millions of years.

Natural Enemies and Niches?

McKenna and Farrell (5) posit that new niches were crucial to the radiations that they demonstrate. If *Cephaloleia*'s parasitoids and predators have been as powerful throughout the Cenozoic as they are now, could not these natural enemies have had a hand in defining niches and affecting diversification? While “top-down” forces are known to be hugely important in protecting tropical plants from insect herbivory (14), “enemy-free space” (15) has been subsidiary to allelochemistry, host plant speciation (5, 16), and geographical separation (5) in theories of speciation. Is it possible that temporary enemy-free space was found by *Cephaloleia* upon new host taxa? Higher population density in the absence of enemies could then have led to greater dispersal and increased geographical range. Subsequent discovery by the enemies and spread through the new larger *Cephaloleia* range could have decreased densities and promoted isolation of populations. The reductions in gene flow ensuing among the smaller and isolated populations could then have promoted differentiation.

McKenna and Farrell (5) have presented one of the most detailed stories of generation, accumulation, and maintenance of Cenozoic diversity to date, a story with substantial ecological and evolutionary implications. We end on a sad note, however. The forests supporting the host plant populations of Middle America that have generated this remarkable efflorescence of *Cephaloleia* diversity over the last 65 million years are being rapidly reduced (17). Many of these beetle species probably face extinction in coming decades.

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