"Inordinate Fondness" Explained: Why Are There So Many Beetles?

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Science **281**, 555 (1998);
DOI: 10.1126/science.281.5376.555

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

tions in all visual cortical areas could underlie a three-dimensional spatial code for addressing and binding of computations carried out in different cortical compartments.

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9. Recording chambers were positioned to permit access to foveal and perifoveal V4 as well as V1 and V2. Two macaque monkeys were trained to reliably fixate a small spot on a computer monitor for a juice reward, and fixation was monitored monocularly with a noninvasive infrared video-based eye tracker [J. Barbur, W. Thomson, P. Forsyth, *Clin. Vision Sci.* **2**, 131 (1987)].
10. The computer monitor was on a movable platform that could be set at 22.5, 45, 90, 180, or 360 cm from the monkey. Interleaved blocks of trials were obtained at three to five of the viewing distances with multiple blocks at each distance. Stimuli were presented in blocks consisting of randomly interleaved presentations of bars of varying size (aspect ratio, 4:1 or 8:1) and scaled with distance so that the bar size was of fixed retinal image size (lengths: 0.2, 0.4, 0.8, 1.6, and 3.2°). At 22.5 cm, the smallest bar (0.2°) was omitted, and at 360 cm, the largest bar (3.2°) was omitted because of physical limitations of the monitor and pixel size. Stimulus intensity was 160.96 ± 3.4 cd · m^{-2}. The bars were swept over the receptive field during fixation at the preferred orientation, direction, and color for the cell. Speed and length of excursion were scaled proportionally with distance to keep retinal speed and excursion constant.
11. Distance modulation and disparity modulation are distinct properties, therefore we use the terms "nearness" and "farness" to distinguish monotonic distance modulation from cells showing near and far binocular disparity-tuning as described by G. F. Poggio and B. Fischer [*J. Neurophysiol.* **40**, 1392 (1977)]. Classification of cells as monotonic (nearness or farness) is not completely certain, because a maximum or minimum could conceivably occur at an unsampled distance. A study of distance and disparity in V1 appears to show that for disparity-selective cells, farness cells are more common than nearness cells (*6*), but differences in stimuli, methods, and analysis preclude direct comparison with our results.
12. Tuning for absolute distance (at least for nearness) has been reported in the ventral intraparietal area of posterior parietal cortex [C. L. Colby, J. R. Duhamel, M. E. Goldberg, *J. Neurophysiol.* **69**, 902 (1993)] and in ventral premotor cortex [M. Gentilucci *et al.*, *Exp. Brain Res.* **50**, 464 (1983); L. Fogassi *et al.*, *J. Neurophysiol.* **76**, 141 (1996)]. However, a study that manipulated viewing distance and binocular disparity in V1 did not find the systematic shifts in preferred disparity with viewing distance that absolute distance tuning would predict (*5, 6*). Moreover, a cell tuned to an intermediate absolute distance would not show monotonic response with viewing distance, as the majority of cells here do. Nonmonotonic cells could be tuned for absolute distance, but these cells made up only 13% of our sample.
13. Cells were assigned to a visual cortical area based on receptive field position, size, and properties, and position relative to the lunate sulcus. Uncertainty about whether certain cells were in V1 or V2 led us to combine V1 and V2 for quantitative analysis.
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16. To ensure that ocular artifacts were not significant, a number of precautions were taken. Both monkeys were refracted by an optometrist using slit retinoscopy to establish that they were capable of accommodation over the range of distances used in the experiment (uncertainty <0.25 diopters). During the experiments, the monitored eye varied its position with distance consistent with the appropriate change of vergence. Pupil radius was measured with the eye tracker and did not vary with distance in either monkey (2.33 ± 0.01 mm; 1.73 ± 0.02 mm). The monkeys were required to maintain fixation within a 0.25° square fixation window during the trial.
17. If viewing distance affected neural response, the measurements were repeated under either binocular or monocular restricted-field viewing conditions. Measurements were then repeated under the initial viewing conditions. The monkey viewed the stimuli through either monocular or binocular apertures (6.5° diameter). The remainder of the scene was masked such that only the monitor screen was visible to the monkey.
18. Because all receptive fields were in or close to the fovea (<2.5° eccentric in all cases), horizontal disparity of stimuli relative to the fixation point would be expected to be very close to zero at all distances. However, if the monkeys made vergence errors during fixation that varied systematically with distance, the responses of disparity-selective neurons could vary with viewing distance during binocular viewing. In the absence of binocular disparity, this argument does not apply, and 15 of 33 neurons maintained distance modulation under monocular restricted-field viewing, demonstrating that distance modulation cannot be attributed to fixation-induced disparity. An independent line of evidence on this point is provided by the modulation of spontaneous activity observed in the absence of a stimulus in half the neurons studied (88/178, $P < 0.01$).
19. For this cell, manipulating the frame size had no effect (Fig. 2E; see figure legend for details), ruling out a center-surround artifact. Local image variations with viewing distance, such as slight changes in brightness or contrast, or changes in pixellation, are common to all the viewing conditions and cannot account for the difference between full-field and restricted-field responses. Nor can fixation disparity-induced horizontal disparity be responsible, because distance modulation is not dependent on binocular viewing. Therefore, local image variation with viewing distance cannot account for distance modulation.
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24. We thank A. Leonardo for contributions to the experiments; E. Dobbins, M. Lewicki, J. Mazer, and D. Rosenbluth for reviewing the manuscript; T. Annau, M. Lewicki, and J. Mazer for assistance with software tools; R. Desimone for providing data collection software; T. Joe for optometric assistance; and H. Weld and J. Baer for veterinary care. All methods of animal care conform to the guidelines of the Caltech Institutional Animal Care and Use Committee and the NIH.

19 February 1998; accepted 8 June 1998

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The phylogeny of the Phytophaga, the largest and oldest radiation of herbivorous beetles, was reconstructed from 115 complete DNA sequences for the 18*S* nuclear ribosomal subunit and from 212 morphological characters. The results of these analyses were used to interpret the role of angiosperms in beetle diversification. Jurassic fossils represent basal lineages that are still associated with conifers and cycads. Repeated origins of angiosperm-feeding beetle lineages are associated with enhanced rates of beetle diversification, indicating a series of adaptive radiations. Collectively, these radiations represent nearly half of the species in the order Coleoptera and a similar proportion of herbivorous insect species.

When the British biologist J. B. S. Haldane was asked by a group of theologians what one could conclude as to the nature of the Creator from a study of His creation, Haldane is said to have answered, "An inordinate fondness for beetles" (*1*). Haldane's remark reflects the numerical domination of described species by the insect order Coleoptera (*2*), the diversity of which exceeds that of any other known animal or plant group. Because over half of all beetles are herbivorous and because the diversity of the remainder is comparable to that of other large, young, and nonherbivorous insect orders (*3*), a reconstruction of the phylogenesis of beetle herbivory would contribute substantially to an understanding of

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possible reasons for the apparent success of the Coleoptera.

Most phytophagous beetles feed on angiosperms, which are the most diverse group of vascular plants. Although the diversity of insects and angiosperms has been thought to result from the interaction of these two groups (*3*), the impact that the rise of flowering plants had on insect diversification has been recently challenged (*4*) by evidence that the appearance rate of insect families did not increase with angiosperm radiation during the Cretaceous. Indeed, most insect families that contain present-day associates of flowering plants were in place by the Jurassic (*5*), with the origins of actual angiosperm associations following later. The most direct test of the influence of flowering plant diversity on insect diversity must evaluate insect diversification rates before and after the origins of associations with angiosperms and must examine diversity within insect families. Phytophagous beetles are critical subjects for these tests, not only because they represent much of the diversity that must be explained, but also because several lineages of phytophagous beetles have colonized angiosperms independently.

Plant feeding arose early in beetle history, about 50 million years after the origin of the Coleoptera in the Permian (*5*). Herbivorous species doubled beetle diversity by the mid-Jurassic and overshadowed the nonherbivorous taxa by the beginning of the Tertiary; this interval coincided with the rise of angiosperms (Fig. 1). The most successful insect-angiosperm associations involve the beetle sister superfamilies Chrysomeloidea and Curculionoidea. These comprise the Phytophaga clade and likely exceed 135,000 species (*6*) [~80% of herbivorous beetles and ~50% of herbivorous insects (*3*)]. The Curculionoidea superfamily consists of six relatively depauperate families (Nemonychidae, Anthribidae, Attelabidae, Belidae, Brentidae, and Rhynchophoridae) and the considerably more diverse Curculionidae, whereas the Chrysomeloidea superfamily consists of the species-rich Cerambycidae and Chrysomelidae families. This assemblage of families contains different lineages, which are associated with cycads or conifers or with monocots or dicots (*7, 8*).

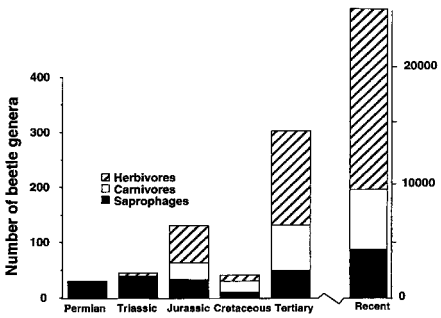

Fig. 1. The number of beetle genera of each of three trophic levels (*34*) per geological period (Permian to Tertiary) and epoch (Recent) (*5, 35*). Permian fossils are entirely of the saprophagous Archostemmata (*5*), and the first Adephaga and Polyphaga (the curculionoid Obrienidae) appear in the Triassic (*9*). Low diversity in the Cretaceous likely reflects the paucity of studied strata. The proportions of fossil genera in each beetle series (defined by Crowson) in the Tertiary and Recent are significantly correlated ($P = 0.001$). The disproportionate rise in the diversity of the post-Cretaceous phytophagous beetles likely reflects the exponential rise in angiosperm diversity, particularly of herbaceous taxa.

The ancestor of the Phytophaga existed ~230 million years ago in the Triassic, as evidenced by the fossils of the now-extinct curculionoid family Obrienidae (*9*). However, the most important Mesozoic strata for fossil weevils and chrysomelids are the Jurassic Karatau beds in Kazakhstan (*10*). These beds contain no angiosperms but are rich in remains of Pteridophyta, Ginkgoales, Gnetales, Coniferales (that is, Araucariaceae and Podocarpaceae), Cycadales, and now-extinct Bennettitales (*10*). The angiosperm and phytophagous beetle fossil records are richest in the post-Jurassic Period, with most of the currently dominant subgroups of monocots and dicots and their herbivores proliferating in the early Tertiary. Because the diversification of seed plants and beetle herbivores has been at least broadly contemporaneous, it is plausible that this history has determined, at least in part, present-day beetle associations and diversity.

To resolve the diversification history of these beetles, DNA sequences for the entire 18*S* ribosomal subunit gene were produced for samples of 115 species, which were drawn from all beetle subfamilies, representing the major variations in host-plant affiliations (*11*). These data were complemented by the addition of a matrix of 212 morphological characters compiled from recent reviews (*12, 13*).

The most parsimonious trees (*14*) (Fig. 2) showed basal conifer- and cycad-feeding beetle lineages in the Chrysomeloidea and Curculionoidea branches. The Chilean *Araucaria*-feeding nemonychid subfamily Rhinorhynchinae [represented by *Mecomacer* (*15*)] is at the base of the Curculionoidea (Fig. 2A), whereas the *Araucaria*-feeding Palophaginae [represented by *Palophagoides* (*16*)] subtends the basal branch in the Chrysomeloidea (Fig. 2B). Immediately following these first branches of the Curculionoidea and Chrysomelidae are branches leading to the Araucariaceae-associated Oxycoryninae [*Oxycraspedus* (*17*)] and Orsodacninae [*Orsodacne* (*18*)] and their respective cycad-feeding sister groups Allocoryninae [*Rhopalotria* (*19*)] and Aulacoscelidinae [*Aulacoscelis* (*20*)]. Similarly, within the Cerambycidae family, the conifer-affiliated Aseminae (*Asemum*) and Spondylinae (*Spondylis*) (Fig. 2B) are the most basal live-plant feeders. All described larvae of these taxa feed on internal host tissues; the feeding of these chrysomelid and curculionoid larvae on the male pollen-bearing strobili of conifers and cycads suggests that attack on these nutrient-rich reproductive structures preceded foliage feeding.

The current affiliations of these oldest beetle lineages with pre-angiosperm seed plants support the hypothesis that these lineages retain affiliations that were formed early in the Mesozoic, before the diversification of flowering plants. Also supportive of early Mesozoic origins are the south temperate distributions of the basal curculionoids and chrysomelids, which are relictual and represent a broader previous distribution on Gondwanaland, before the late Mesozoic breakup (*21*). Thus, the evidence from phylogenetic position and biogeography points to the con-

Table 1. Five independent contrasts of groups associated with gymnospermous seed plants versus angiosperms. All five contrasts yield a positive difference in favor of the hypothesis that angiosperm feeding is associated with enhanced diversity (one-tailed sign test, $P = 0.03$). Addition of the remaining (mostly weevil) subfamilies, not yet sequenced, will bring the total number of species to 135,000. For two comparisons, alternative topologies are three to four steps (combined changes in nucleotides and morphological characters) away (comparisons 3 and 5), but these alternatives yield the same conclusion of ancestral beetle associations with gymnosperms. Thus, for comparison 3 (the Cerambycidae), the closest alternative grouping (within four steps) is of the Spondylinae as sister to the angiosperm-associated clade, with Aseminae as sister to this assemblage. For comparison 5, the closest alternative (within three steps) is of Orsodacninae as sister to the angiosperm feeders.

Comparison	Primitively gymnosperm-associated taxon	Diversity	Primitively angiosperm-associated taxon	Diversity
1	Nemonychidae	85	Attelabinae-Rhynchitinae, Apioninae, and Curculionidae-Rhynchophoridae	44,002
2	Oxycoryninae-Allocoryninae	30	Belinae	150
3	Aseminae-Spondylinae	78	Lepturinae and Lamiinae-Cerambycinae	25,000
4	Palophaginae	3	Megalopodinae-Zeugophorinae	400
5	Orsodacninae-Aulacoscelidinae	26	Remaining Chrysomelidae	33,400

clusion that these associations of beetles with conifers and cycads are nearly 200 million years old and are therefore the oldest extant insect-plant interactions known.

The phylogenetic ordering of beetle-plant associations is borne out by the concordant stratigraphic distributions of taxa in the two groups. The nemonychid subfamily Rhinorhynchinae (*22*), the belid subfamily Oxycoryninae (*23, 24*), and the chrysomelid subfamily Palophaginae (*25*), all of which attack the male strobili of *Araucaria*, contain members that are found in Kazakhstan in the Jurassic Karatau Formation, in which *Araucaria* fossils are prominent. The Araucariaceae show remarkable continuity between Mesozoic and extant forms, because Jurassic fossil cones and leaves are attributable to extant sections of *Araucaria* (*26, 27*). Indeed, the investment of fossil and extant *Araucaria* reproductive parts with defensive resin canals supports an argument for the early and continued vulnerability of *Araucaria* to herbivorous insects (*28*). The discovery of extremely well preserved *Araucaria* strobili (some with apparent beetle damage) and foliage in the Jurassic fossils of Argentina suggests that these Argentine beetles may have been continuously associated with their hosts in a single place. Such continuity in insect associations therefore extends the morphological continuity of the Araucariaceae to include ecological interactions with herbivores.

Some present-day cycad associates predate the rise of angiosperms. The phylogeny estimate predicts the early appearance of the cycad-feeding beetle subfamilies Allocoryninae and Aulacoscelinae, insects that are found in the Jurassic Karatau beds (*29, 30*). The pairing of the cycad-feeding taxa with associates of Araucariaceae in both the Chrysomeloidea and Curculionoidea apparently reflects the codominance of these Late Jurassic flora members and also reflects, perhaps, the nutritional similarity of their relatively large male strobili (*31*).

Although the fidelity of the oldest beetle-host associations might reflect features of conifers and cycads (or features of these particular beetles) that promote their stability, many angiosperm-affiliated beetle subfamilies or tribes are restricted to taxonomic groups of monocots or dicots as well (Fig. 2). The persistent affiliations of beetle clades with plants that represent the range of potential host groups that formed throughout the latter half of the Phanerozoic Eon clearly impose a strong imprint of evolutionary history on the structure of modern insect-plant communities and thereby bear implications for their relative diversity.

The phylogeny estimate permits a test of the hypothesis that proposes that the angiosperm-feeding origins in the beetles are associated with enhanced diversity. To apply this estimate, the diversity of each group for which angio-

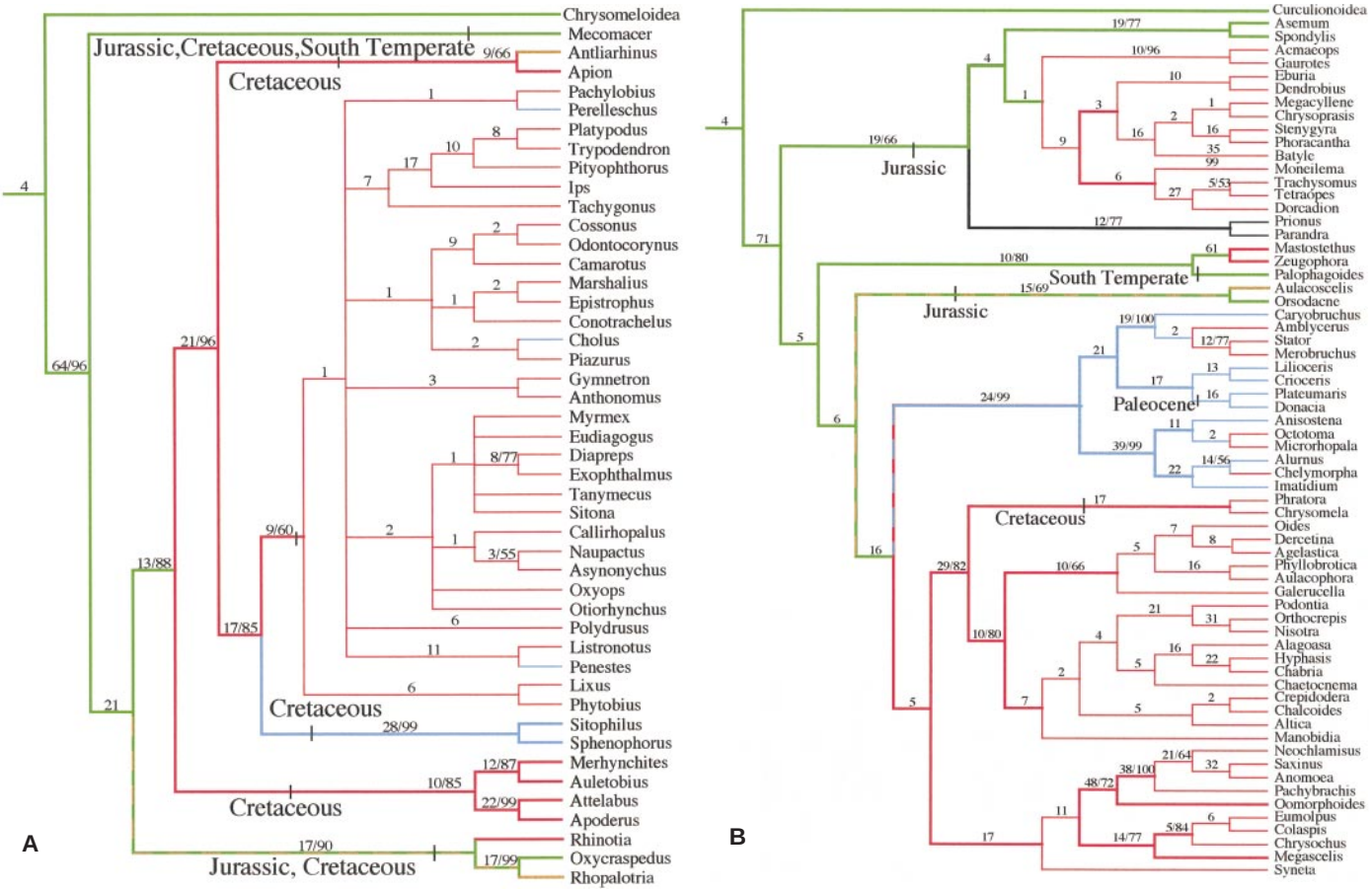

Fig. 2. Estimate of the phylogeny of host associations in the Phytophaga, on the basis of simultaneous analyses of DNA sequences and morphological characters for (**A**) Curculionoidea, (**B**) Chrysomeloidea, and outgroups. The strict consensus tree for the two superfamilies, minus outgroups, is presented in two parts for legibility, with numbers indicating the number of synapomorphies/only those bootstrap values that exceed 50% (length, 2086; consistency index, 0.5; rescaled consistency index, 0.4; retention index, 0.83). Individual numbers also represent the number of synapomorphies. The Phytophaga, Chrysomeloidea, and Curculionoidea are all monophyletic, and the erotylid and melyrid sequences form the sister group to the Phytophaga, with *Tenebrio* outside these. Common groups between separate analyses of DNA sequences and morphological characters are represented by bold lines (DNA sequences are the sole source of resolution below the subfamily level in the Chrysomeloidea and below the family level in the Curculionoidea). Colors indicate the major host group attributable to the common ancestor of each group (green, Coniferae; brown, Cycadales; red, dicotyledonous angiosperms; blue, monocotyledonous angiosperms; black, subfamilies that do not feed on living plants). Approximate ages of Mesozoic and early Tertiary fossils only are indicated where known, because almost all subfamily groups are known from the mid-Tertiary fossil record.

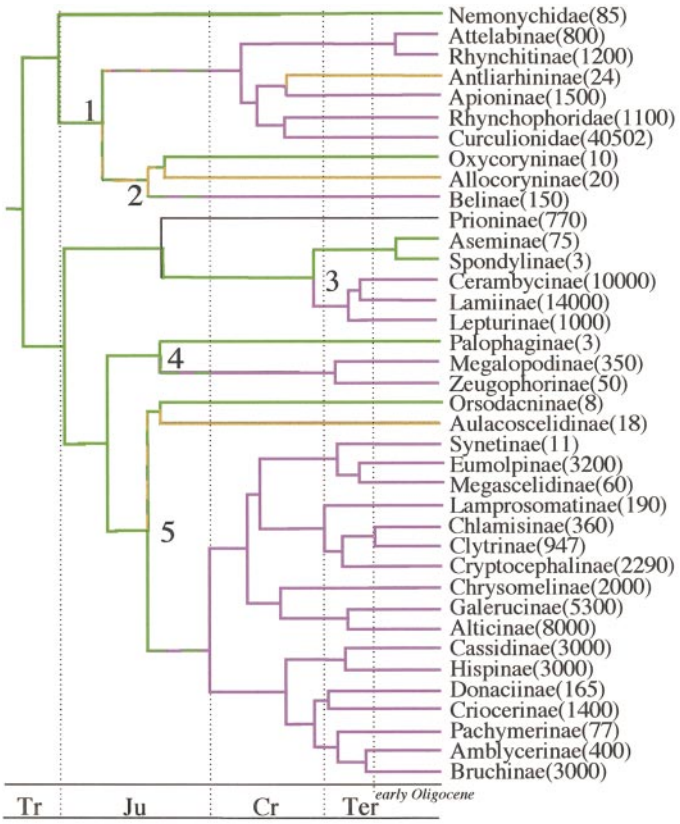

Fig. 3. The phylogeny of the families and subfamilies of Phytophaga represented by genera in Fig. 2, with estimates of the number of current species in parentheses (*36*). Branches are colored by major host-plant group as in Fig. 2, but with purple indicating the collective use of angiosperms. The approximate age of each clade (estimated from the beetle fossil record) is indicated by the depth of the branches, with dotted lines superimposed for each period. The five origins of associations with angiosperms are numbered. In the Curculionoidea, an equally parsimonious interpretation would be an origin of angiosperm association at 1 followed by a reversal to cycad-*Araucaria* association at 2. However, this interpretation seems less plausible than two separate origins in the Cretaceous, because angiosperms were not developed in the Jurassic (*37*).

sperm association was clearly the ancestral habit it was contrasted with the diversity of the respective sister group for which cycad feeding or conifer feeding was clearly ancestral (Fig. 3). This analysis identified five such contrasts (Table 1), all of which show an increased diversity (of several orders of magnitude) in the angiosperm-associated group (one-tailed sign test, $P = 0.03$). The total increase in beetle diversity is ~100,000 species, which is directly attributable to a series of adaptive radiations onto angiosperms.

The diversification of the phytophagous beetles is consistent with the coevolutionary model of Ehrlich and Raven (*32*), who ascribe differences in the present diversity of insect and plant groups to evolutionary changes in characters (which affect their ecological interactions) and who predict that older plants should harbor older herbivores. Combined evidence from the phylogeny estimates presented here and from the fossil record shows a pronounced conservatism in the evolution of beetle-plant associations, which is important for the implication that plants might escape herbivory via key innovations (*28, 32*). Correlated with angiosperm feeding is the proliferation of life-history traits in the curculionids and chrysomelids. In contrast with the strobilus feeding of conifer- and cycad-associated ancestors, diversification of the subfamilies that attack flowering plants has been accompanied by larval folivory, leaf mining, and seed and root feeding, which exemplify the concept of adaptive radiation.

Although Haldane's remark reflected a common and understandable emphasis on explaining the diversity of a particular taxon, explanations may be more readily found through comparative investigations of ecological breakthroughs that have evolved sufficiently often to permit multiple comparisons to be made (*33*). The success of the order Coleoptera thus seems to have been enabled by the rise of flowering plants.

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11. Beetle groups that are restricted to particular higher plant taxa were scored for the least inclusive plant taxon that contained their hosts. The cerambycid subfamilies Prioninae and Parandrinae were left unscored for host, as these do not feed on live plant tissues but on dead or decaying wood [E. G. Linsley, *Univ. Calif. Berkeley Publ. Entomol.* **18**, 1 (1961)]. The only higher beetle taxa for which DNA sequences were not obtainable were the Sagrinae and Anthribidae.
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14. Sequences were obtained for the complete 18*S* ribosomal subunit gene from 115 of these beetle taxa and from the outgroup species from the Tenebrionidae (*Tenebrio molitor*, GenBank accession number 70810), Melyridae (*Collops quadrimaculatus*), and Erotylidae (*Cypherotylus boisduvali*) with methods that were given by M. F. Whiting, J. C. Carpenter, Q. D. Wheeler, W. C. Wheeler, *Syst. Biol.* **46:**, 1 (1997). These sequences were aligned using Sequencher 3.0 (Gene Codes Corporation Ann Arbor, MI, 1995), producing a matrix of 2117 positions. Three ~40–base pair (bp), hypervariable regions could not be unambiguously aligned and were excluded from the analyses, as were the two 50-bp ends of the gene, to avoid excessive missing data in parts of the matrix. The remaining 1874 positions yielded 355 potentially informative characters. These characters were analyzed separately and together with the morphological matrix compiled from Kuschel and Reid (*12, 13*). Analyses using the program PAUP* 4.0 version d59 included 100 initial heuristic searches using random taxon addition sequences and tree bisection-reconnection (TBR) branch swapping, setting MAXTREES (maximum number of trees held in memory) to 200, and keeping two trees per replicate search. This set of 200 trees was then subjected to TBR branch swapping with MAXTREES set to 10,000. Bootstrap analysis used 1000 random taxon addition sequences, with branch swapping limited to 100 trees per replicate. Tests of incongruence (using simple addition sequences and limiting MAXTREES to 100) between morphological and molecular data sets were not significant (incongruence length difference, $P > 0.5$).
15. The Rhinorhynchinae subfamily includes the most morphologically plesiomorphic nemonychids, and they currently consist of 14 genera associated with strobili of Araucariaceae or Podocarpaceae in Chile, Argentina, and Australia plus a single species living on Pinaceae in Colorado [G. Kuschel, *Rev. Chil. Hist. Nat.* **54**, 97 (1954)]. The closely related Holarctic Doydirhynchinae comprise 19 species living on Pinaceae. Crowson removed the nominate genus *Nemonyx* to the Anthribidae [R. A. Crowson, *Entomol. Mon. Mag.* **121**, 144 (1985)].
16. The Palophaginae consist of three species in two genera, which develop in the male strobili of Araucariaceae in Chile, Argentina, Australia, and New Zealand [G. Kuschel and B. M. May, *N. Z. Entomol.* **19**, 1 (1996)].
17. The most plesiomorphic oxycorynine belid genus *Oxycraspedus* attacks *Araucaria* strobili in Chile and Argentina [G. Kuschel, *Invest. Zool. Chil.* **5**, 229 (1959)]. Crowson also suggested that *Oxycraspedus* and *Rhopalotria* are sister taxa but did not place the morphologically disparate oxycorynine genera reported from the Hydnoraceae and Balanophoraceae, which are families of tree parasites [R. A. Crowson, in *Advances in Coleopterology*, M. Zunino, X. Belles, M. Blas, Eds. (European Association of Coleopterology, Barcelona, 1991), pp. 13–28]. The belid tribe Pachyurini comprises 13 genera associated with *Araucaria* and *Agathis* in Australia and New Zealand and a single genus associated with Podocarpaceae and Cupressaceae in Brazil.
18. The Orsodacninae comprise the Australian genus *Cucujopsis*, which is associated with the male strobili of the araucariaceous genus *Agathis* and the Holarctic

genus *Orsodacne* [J. S. Mann and R. A. Crowson, *J. Nat. Hist.* **15**, 727 (1981)]. Although the larval affiliations of *Orsodacne* are still unconfirmed, these are probably in the male strobili of Pinaceae (with which all eight species co-occur), a resource available during the early spring flights of the pollen-feeding adults.
19. The belid subfamily Allocoryninae comprises >20 species in the Neotropical genus *Rhopalotria*, which attack the male strobili of *Zamia* and *Dioon*.
20. The chrysomelid subfamily Aulacoscelidinae comprises 18 species in two Neotropical genera restricted to the Cycadaceae.
21. L. Brundin, *Evolution* **19**, 496 (1965).
22. G. Kuschel, in *Australian Weevils*, E. Zimmerman, Ed. [Commonwealth Scientific and Industrial Research Organization (CSIRO), Melbourne, Australia, 1994], p. 569. Other nemonychids in the Karatau Formation apparently belong to the now-extinct subfamily Brenthorrhininae (*9*). The Nemonychidae are also represented by *Libanorhinus succinus* in Lower Cretaceous amber derived from Araucariaceae resins [G. Kuschel and G. O. Poinar, *Entomol. Scand. (Group 2)* **24**, 143 (1993)] and by the Lower Cretaceous *Slonik* in the central Asian trans-Baikal deposits [G. Kuschel, *GeoJournal* **7**, 499 (1983)].
23. The oxycorynine *Archeorrhynchus paradoxopus* (Belidae) is found in the Karatau Formation [G. Kuschel, in *Australian Weevils*, E. Zimmerman, Ed. (CSIRO, Melbourne, Australia, 1994), p. 244]. Oxycoryninae are also represented in the Lower Cretaceous Santana Formation of Brazil [D. A. Grimaldi, Ed., *Bull. Am. Mus. Nat. Hist.* **195**, 8 (1990)]. Additional Karatau belids include the extinct subfamily Eobelinae [V. V. Zherikhin and V. G. Gratshev, in *Biology and Classification of Coleoptera: Papers Celebrating the 80th Birthday of Roy A. Crowson*, J. Pakaluk and S. A. Slipinski, Eds. (Muzeum I Instytut Zoologi PAN, Warsaw, 1995), p. 646].
24. The belid subfamily Carinae, which attacks strobili of the coniferous Cupressaceae, occurs in the Jurassic Karatau beds, as represented by *Eccoptarthrus* and *Emanrhynchus* [V. V. Zherikhin and V. G. Gratshev, in *Biology and Classification of Coleoptera: Papers Celebrating the 80th Birthday of Roy A. Crowson*, J. Pakaluk and S. A. Slipinski, Eds. (Muzeum I Instytut Zoologi PAN, Warsaw, 1995), pp. 634–777]. The Carinae also appear in the Lower Cretaceous trans-Baikal beds (*Cretonanophyes* and Baissorhynchus); the Carinae presently contains *Car*, which is found in Australia and Tasmania, and *Chilecar* and *Caenominurus*, which are found in Chile and Argentina [E. Zimmerman, Ed., *Australian Weevils* (CSIRO, Melbourne, Australia, 1994), p. 504.
25. The chrysomelid *Cerambyomima longicornis*, attributed to the Aulacoscelinae [G. Kuschel and B. M. May, *Invertebr. Taxon.* **3**, 697 (1993)], resembles the orsodacnine *Cucujopsis* in the grooved frons and may be an intermediate form.
26. Jurassic fossil cones of *Araucaria mirabilis* from Argentina closely resemble *A. bidwellii* and show damage similar to that caused by weevil larvae [see R. A. Stockey, *Paleontographica* **166**, 1 (1978)]. *A. bidwellii* is host to extant species in both the Nemonychidae and Palophaginae.
27. R. A. Stockey, *J. Plant Res.* **107**, 493 (1994).
28. B. D. Farrell, D. Dussourd, C. Mitter, *Am. Nat.* **138**, 881 (1991).
29. The allocorynine *Scelocamptus curvipes* is found in the Karatau beds (*10*).
30. The aulacosceline genera *Protoscelis*, *Protosceloides*, and *Pseudomegamerus* are found in the Karatau beds (*5*).
31. T. N. Taylor and E. L. Taylor, *The Biology and Evolution of Fossil Plants* (Prentice-Hall, Englewood Cliffs, NJ, 1993).
32. P. R. Ehrlich and P. H. Raven, *Evolution* **18**, 586 (1964); B. D. Farrell and C. Mitter, *Biol. J. Linn. Soc.* **68**, 533 (1998).
33. J. Jernvall, J. P. Hunter, M. Fortelius, *Science* **274**, 1489 (1996).
34. Assignments of feeding habits and numbers of recent genera are from Lawrence (*6*).
35. The number of genera was extracted from the totals per beetle family in Lawrence (*6*).
36. Estimates of diversity are from the following sources: Curculionoidea (*7*); Chrysomelidae [P. Jolivet, E. Petitpierre, T. H. Hsiao, Eds., *Biology of Chrysomelidae* (Kluwer Academic, Dordrecht, Netherlands, 1988)]; Cerambycidae [S. Bily and O. Mehl, *Longhorn Beetles (Coleoptera, Cerambycidae) of Fennoscandia and Denmark*, vol. 22 of *Fauna Entomologica of Scandinavica* (Brill, Leiden, Netherlands, 1989)].
37. For a discussion of the use of fossils to assign character optimizations, see J. M. Doyle and M. J. Donoghue, *Rev. Palaeobot. Palynol.* **50**, 63 (1987).
38. For supplying specimens or identifications of key or austral taxa, I especially thank F. Andrews, J. Chemsak, L. Diego-Gomez, J. Donaldson, C. Duckett, T. Erwin, W. Flowers, D. Furth, C. D. Johnson, J. Kingsolver, G. Kuschel, J. Lawrence, A. Newton, K. Norstog, R. Oberprieler, C. O'Brien, and E. G. Riley, among many others. I also thank A. Salmore, M. Blair, and L. Morrissey for technical lab support; A. Berry, M. Donoghue, D. Futuyma, A. Knoll, D. Lewontin, E. Mayr, C. Mitter, N. Moran, B. Normark, S. Palumbi, N. Pierce, and E. O. Wilson for helpful discussions; and A. Knoll, C. Labandeira, and D. Maddison for detailed comments on a late draft. This research was supported by NSF, USDA, and the Putnam Expedition Fund of the Museum of Comparative Zoology.

19 January 1998; accepted 8 June 1998

Activity-Dependent Cortical Target Selection by Thalamic Axons

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Connections in the developing nervous system are thought to be formed initially by an activity-independent process of axon pathfinding and target selection and subsequently refined by neural activity. Blockade of sodium action potentials by intracranial infusion of tetrodotoxin in cats during the early period when axons from the lateral geniculate nucleus (LGN) were in the process of selecting visual cortex as their target altered the pattern and precision of this thalamocortical projection. The majority of LGN neurons, rather than projecting to visual cortex, elaborated a significant projection within the subplate of cortical areas normally bypassed. Those axons that did project to their correct target were topographically disorganized. Thus, neural activity is required for initial targeting decisions made by thalamic axons as they traverse the subplate.

During the wiring of connections between the thalamus and cortex in mammals, there is an intermediate step in which thalamic axons grow and interact with a special population of neurons—subplate neurons—before they contact their ultimate target neurons within the cortical plate (*1*, *2*). For example, LGN axons en route to visual cortex emit transient side branches that extend into the subplate under both target and nontarget cortical areas (*3*) and form functional synaptic contacts with subplate neurons (*4*). During this period of development, spontaneous action potential activity generated in the retina and relayed through the LGN likely drives these subplate synapses in vivo (*5*). Thus, synaptic relations within the subplate could support activity-dependent interactions during the process of thalamocortical axon target selection.

To examine if activity is needed for thalamic axons to form connections with their appropriate cortical target area, we infused tetrodotoxin (TTX, a sodium channel antagonist that blocks action potentials) or vehicle through osmotic minipumps (*6*) into the brain of cat fetuses between E42 (E42 = 42 days of gestation) and E56. At E42, the first LGN axons have just reached the subplate underneath visual cortex but still have side branches along their trajectory. Between E42 and E50, the majority of LGN axons have arrived in the visual subplate; by E56, many have departed the subplate and reached their ultimate target, layer 4 of the cortical plate (*3*). To assess the consequences of the treatments on the thalamocortical projection, we injected carbocyanine dyes at E56 to label retrogradely LGN neurons (*7*) and subsequently counted the numbers of neurons sending axons to the subplate or cortical plate of either visual (the correct target) or auditory (an incorrect target) cortex.

The number of LGN neurons projecting to visual cortex was decreased in TTX-infused animals (Fig. 1), both within the subplate [Fig. 1C; an average of 69 ± 5% SEM fewer neurons than vehicle controls, $n = 8$ animals; 4 littermate pairs treated with TTX or vehicle and matched for similar 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) injection sizes] and within the cortical plate (Fig. 1C; 94 ± 0.5% SEM, $n = 8$ animals; 4

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