the important functions of setting the resting potential of the cell and repolarizing the membrane after an action potential (see figure, part A). With no structural or chemical evidence to guide them, they said little about the nature of the conducting path. Subsequently, Hodgkin and Keynes (*12*) described a "long pore" effect for K$^+$ channels, which suggested that two or more K$^+$ ions at a time permeate in single file. This general idea fit well with results from experiments with the cation tetraethylammonium (TEA$^+$) and its derivatives (*13*). TEA$^+$—a central nitrogen surrounded by four ethyl arms—is about 8 Å in diameter, approximately the size of K$^+$ with one hydration shell. TEA$^+$ applied to the intracellular side of the pore blocks K$^+$ channels, and the internal receptor is protected by a gate that must be open for TEA$^+$ to enter. A simple picture is that both TEA$^+$ and a hydrated K$^+$ ion (see figure, B) can occupy the channel's inner vestibule, but only the K$^+$ ion can pass through a narrow part, by partially dehydrating. TEA$^+$, with covalently linked ethyls, remains stuck in the vestibule, blocking the pore. The narrow part of the pore was measured by Hille with ion substitution experiments (*14*) and is about 3 Å in diameter. Experiments with TEA$^+$ derivatives (for example, C$_9^+$, which has seven carbonyls added to one arm) indicated the presence of a hydrophobic region, now clearly seen in the crystal structure, in the inner vestibule.

But how does a 3-Å pore prevent the permeation of Na$^+$, which is only 1.9 Å in diameter? One thought was that a permeant ion had to fit closely in the pore (*16*). A K$^+$ ion in a pore of fixed 3-Å diameter and coordinated by carbonyl oxygens could have much the same energy as a K$^+$ ion in water (see figure, C and D). For a Na$^+$ ion, the rigid pore will not collapse to form good bonding (see figure, F), making the ion's energy higher than in water (see figure, E). Remarkably, the crystal structure vividly reveals a framework for providing such rigidity, a key point in understanding selectivity.

This work answers in some detail, sufficient for the present, the question of K$^+$ channel selectivity. Inactivation gating is already essentially solved (*15*). Next will come the question of voltage gating of channels, a fascinating property of the more complicated six-transmembrane voltage-gated channels.

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PALEOBIOLOGY

How Old Is the Flower and the Fly?

Conrad C. Labandeira

The first book that Charles Darwin wrote after publication of *The Origin of Species* (1859) was *On the Various Contrivances by Which British and Foreign Orchids Are Fertilised by Insects, and on the Good Effects of Intercrossing* (1862), an intellectual forerunner to modern work on pollination biology. In this volume, Darwin applied a tradition of careful observation with principles such as reciprocal adaptation toward understanding pollination—one of the most pervasive and diverse of mutualisms known in nature. Orchids, although fascinating in their own right and the premiere group of plants renowned for their intimate and intricate coevolutionary associations with pollinating insects (*1*), nevertheless represent a relatively recent (Cenozoic) event in the geologic history of pollination. Recently, Friis (*2*) and others have produced anatomical evidence from spectacularly preserved floral structures and have elucidated the first occurrences of pollinator-associated floral features during the mid-Cretaceous. These angiosperm-centered discoveries have pinpointed some of the earliest known fossil occurrences of particular pollination syndromes. [Pollination syndromes are morphologically convergent adaptive trends exhibited by both the floral features of pollinated plants and the mouthpart structure and other flower-interactive features of their respective pollinators (*1*).] Nevertheless, the earlier Mesozoic history of insect pollination is considerably more ambiguous. At present, there are few clues regarding the pollination biology of "preangiospermous" Mesozoic insects. Most inferences come from modern associations between primitive lineages of insects and their gymnospermous seed plant hosts, especially cycads (*3*), and from fossil gut contents and coprolites of pollen-consuming insects (*4*). Diagnostic mouthpart structures (*4*, *5*) are rarely observed, which is now remedied by the discoveries reported by Ren on page 85 of this issue (*6*). This impressive documentation now places three lineages of lower brachyceran flies [see figure, panel (**B**)] as pollinators in China during the Upper Jurassic. However, as explored below, the group of plants that these external fluid feeders were pollinating is as intriguing as the presence of the pollination itself.

Pollen consumption (pollinivory) has generally been the evolutionary precursor to pollination. Pollinivory can become a mutualism (that is, pollination) if the pollinivore can deliver unconsumed pollen to the female reproductive organs of its host plant more efficiently than alternative dispersal by wind, splashing rain, or gravity. Pollination mutualisms require a plant to sacrifice pollen for improved access and efficiency in the fertilization of conspecific ovules. Even pollinivory is a derived feeding strategy, because it is temporally preceded by spore consumption (sporivory) in the fossil record. The earliest terrestrial sporivory occurs in Late Silurian to Early Devonian terrestrial ecosystems, indicated by distinctive coprolites, produced probably by myriapods or insects, with abundant to occasional spore contents from primitive land plants (*7*) [see assemblage 1 in panel (**A**) of the figure]. During the Carboniferous, a younger assemblage has been documented [assemblage 2 in panel (**A**) of figure], represented by insects. By the end of the period, pollen consumption was established, evidenced both by well-preserved, dispersed coprolites [panels (**D**) and (**E**) of figure] and gut contents of hemipteroid and ortho-

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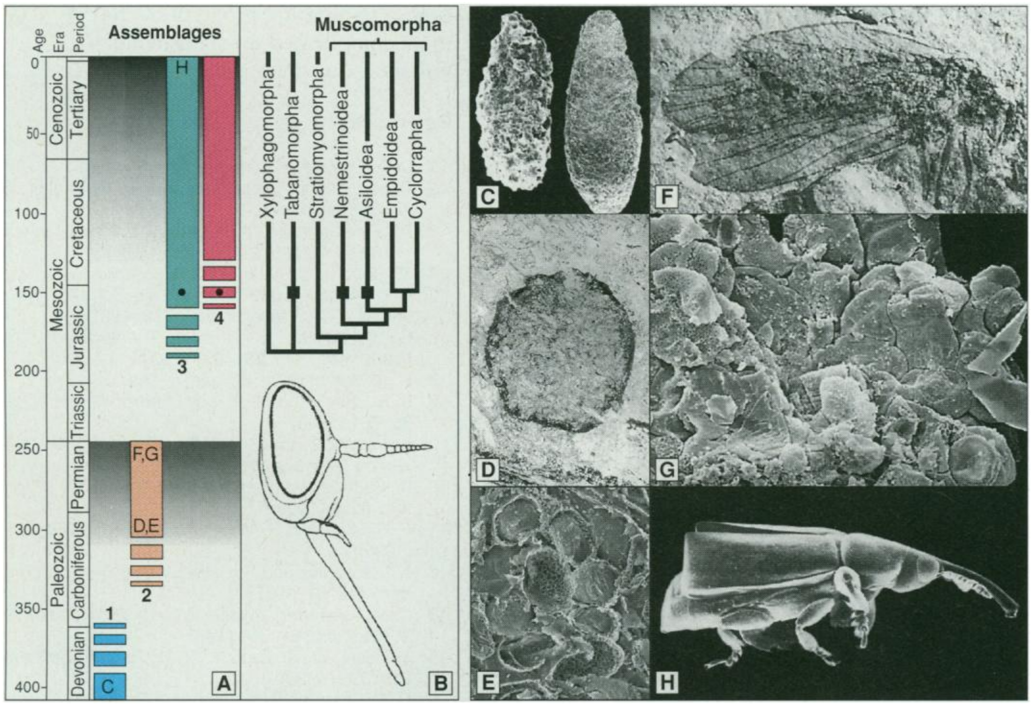

Evolving together. The fossil history of associations between insects and reproductive structures of vascular plants. (**A**) The four distinctive assemblages of fossils (see text for description) representing consumption of spores, pollen, or nectar, based on a variety of evidence, such as the examples at right. The presence and intensity of background shading indicate the probable duration and pervasiveness of pollination. Dots in Late Jurassic denote suites of insects (*5*, *6*) that could be assigned to either assemblages 3 or 4 or both. (**B**) (Top) Cladogram of major subgroups of the lower Brachycera [after (*16*)], showing three fly lineages inferred by Ren (*6*) to have been Late Jurassic angiosperm pollinators. (Bottom) The head and proboscis of an extant Australian member of the Tabanomorpha (*17*). (**C**) Ellipsoidal spore-bearing coprolites from Late Silurian (left) and Early Devonian (right) floras (*7*). The left specimen consists of plant cuticle with occasional spores; the right specimen comprises mostly spores. (**D**) Late Carboniferous insect coprolite from the Illinois Basin, USA, consisting of pollen from a cordaitalean gymnosperm, enlarged in (**E**). (**F**) A Lower Permian hypeperlid insect from Russia, with rectal contents illustrated in (**G**), containing pollen grains from glossopterid and conifer gymnosperms (*8*). (**H**) The snout weevil *Rhopalotria mollis*, pollinivore and pollinator of the extant cycad *Zamia furfuracea* (*3*).

F. MARSH/SMITHSONIAN INSTITUTION; (C) *NATURE* 377, 329 (1995); (F, G) *LETHAIA* 29, 369 (1997); (H) ORGANIZATION FOR TROPICAL STUDIES

pteroid insects (*8*) [panels (F) and (G) of figure]. Coeval pollination mutualisms have been inferred from the reproductive biology of certain seed ferns, such as anomalously large pollen grains, investitures of secretory glands adjacent to reproductive structures, and the presence of pollination drop mechanisms for attracting pollen and potentially insects (*4*). Curiously, elongate mouthparts are known from the Permian (*4*, *8*, *9*), probably representing feeding on surface fluids. The lineages displaying these mutualisms undoubtedly were extinguished during the late Permian; substantial evidence for pollinivory and pollination does not reappear until the Jurassic.

Three diverse lines of evidence currently indicate that basal lineages of modern insect pollinators originated during the Jurassic, probably as generalists on seed plants [assemblage 3 in panel (A) of figure] (*4*, *8*). First, although recently viewed as exclusively wind-pollinated, modern cycads are now considered overwhelmingly insect-pollinated (*3*). Studies now demonstrate beetle pollinivory in 7 of the 10 extant genera of modern cycads, and apparently faithful pollination occurs in those species that have been extensively investigated. These cycad-inhabiting beetle lineages are extant representatives of basal lineages of the Curculionoidea (weevils and relatives) that originated during the Late Jurassic (*10*). This shift in received wisdom has also been demonstrated for a second clade of advanced seed plants, the Ephedrales, of which *Gnetum* and *Ephedra* are now known to be insect-pollinated as well, especially by moths (*11*, *12*). A second line of evidence is fossil evidence for plant damage, including fecal pellets in chambers evacuated within bennettitalean strobili, and coniferalean and ephedralean pollen in the gut contents of orthopteran and holometabolous insects (*4*, *8*). Last, there has been limited evidence for pollen- and nectar-imbibing insect mouthparts that are difficult to explain otherwise. Examples include nemonychid weevils, glossatan moths, and nemestrinid flies (*5*). To this Jurassic list, Ren (*6*) adds evidence of elongate mouthparts and body hair patterns from tabanid, protapiocerid, and additional nemestrinid flies. Although Ren indicates that these findings provide evidence for Late Jurassic angiosperms [initiating assemblage 4 in panel (A) of figure], it is equally likely that basal brachyceran lineages of flies were pollinating anthophytes other than angiosperms, lured by exposed sugary fluids secreted by nectaries located on vegetative or reproductive structures, pollination drop exudates, or even secondarily produced substances such as honeydew from sap-sucking insects (*4*, *12*, *13*). Seed plant candidates include ephedraleans and cycads and extinct clades such as bennettitaleans, corystosperms, and caytonialeans (*1*).

Ren's (*6*) documentation of elongated mouthparts and other pollinator-associated features of Late Jurassic brachyceran flies, bolstered by recent advances in cycad-weevil pollination biology and the record of mid-Mesozoic plant-insect interactions, supports the above hypothesis that the origin of modern lineages of pollinating insects resides amid Jurassic gymnospermous seed plants. If we accept literally the earliest convincing record of angiosperms, well into the Early Cretaceous (*11*), then those lineages of pollinating insects that existed during the later Jurassic may have had their mutualisms subsequently co-opted and fine-tuned by angiosperms. The earliest evidence for fly pollination in angiosperms is during the mid-Cretaceous, among several early lineages of angiosperms bearing small, exposed flowers with relatively accessible floral rewards; however, their floral morphology indicates that their pollinators possessed short, sponging (labellate) mouthparts (*1*, *14*) and that they were well established within assemblage 4 [panel (A) of figure]. Deeper throated flowers that require elongate, probing proboscides are relatively derived in angiosperms (*15*) and appeared later during the Cretaceous (*2*). One instructive counterexample to the above pattern of angiosperm co-optation is beetle pollinators and their cycad hosts, which represent an independent and parallel development that has persisted to the present (*10*).

Consequently, investigations of the origins of basal groups of modern pollinating insects must explore more completely assemblage 3 [panel (A) of figure], of which there is tantalizing but still incomplete evidence. These investigations will require extensive examination of Middle Jurassic to earliest Cretaceous compression deposits. Although there has been considerable effort toward characterizing the insect constituents of Cretaceous amber, the oldest insect-bearing amber is about 125 million years old and thus too recent to address the origin of the basal clades of modern insect pollinators.

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NEUROSCIENCE

Memory and Awareness

Daniel L. Schacter

When we remember our past experiences, we typically invoke a previous conscious awareness of these events. But memory for some aspects of the past can be expressed without any awareness that one is "remembering." These two kinds of memory are described as explicit or declarative memory (when we consciously recollect previous experiences) (*1*) and implicit or nondeclarative memory (when past experiences influence current behavior or performance even though we do not consciously recollect them). Recent advances in cognitive neuroscience are beginning to reveal the brain systems underlying the two forms of memory. Clark and Squire's (*2*) article on page 77 of this issue, which examines classical conditioning in healthy volunteers and amnesic patients, provides a striking example of the role of awareness in remembering that is best understood in the broader context of explicit and implicit forms of memory.

Amnesic patients, who have selective damage to the inner (medial) regions of the temporal lobes (including the hippocampus and related structures) perform poorly on tests for explicit memory that require them to recall or recognize recently presented information. But the same patients often show normal performance on implicit memory tests, in which they are simply asked to carry out a task and are not required to recollect any past experiences (*1*). Consider, for example, a type of implicit memory known as priming: a change in the ability to identify or produce an item as a result of a previous encounter with the item. In tests for priming, participants are asked to complete fragmented words or identify a word or picture after a brief exposure. Priming has occurred when individuals can complete or identify items that they have recently studied faster or more accurately than novel, nonstudied items. Amnesic patients exhibit normal priming effects on a variety of tasks (*3*). Thus, the medial temporal lobe (MTL) regions that are damaged in amnesiacs are crucial for explicit memory but are not needed for priming and related forms of implicit memory (*1*, *3*).

Amnesic patients show normal delay conditioning of an eyeblink response, as reported in previous studies and by Clark and Squire (*2*). This result fits well, because the delay conditioning paradigm does not require any explicit memory. In delay conditioning, participants simply listen to a tone followed immediately by an air puff that elicits an eyeblink response; after a number of such pairings, the tone alone elicits the eyeblink response.

More puzzling are findings from earlier research and from Clark and Squire (*2*) indicating that amnesic patients do not develop normal trace conditioning, which involves the same procedures as delay conditioning with one difference: in delay conditioning the tone and air puff overlap temporally and terminate at the same time, whereas in trace conditioning there is a brief interval after the offset of the tone and onset of the air puff. Why would this brief delay (which falls within the preserved immediate memory span of amnesic patients) produce a conditioning deficit? A key finding from Clark and Squire's new study—that trace conditioning in healthy volunteers occurs only in those who exhibit awareness of the contingency between tone and air puff, whereas delay conditioning occurs independently of such awareness—provides a neat answer. Amnesic patients cannot call on the explicit or declarative memory used by healthy volunteers to develop awareness of the contingency that is necessary for trace but not delay conditioning.

Memory phenomenon	Awareness necessary?	MTL necessary?
Delay conditioning	No	No
Trace conditioning	Yes	No
Word completion priming	No	No
Associative completion priming	Yes	Yes
Sequence learning	No	No

These results may help in understanding data from a recent study (*4*) in which brain activity was examined in healthy volunteers during delay conditioning with positron emission tomography (PET), which provides an index of local neuronal activity by measuring changes in regional cerebral blood flow. The medial temporal lobes were activated during delay conditioning. In light of Clark and Squire's data, it seems likely that this activation is associated with incidental awareness of the tone–air puff relation on the part of some experimental par-

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