Amber's Botanical Origins Revealed

Large collections of the substances exuded by trees today may help to track backward from amber samples to the ancient plants that produced them

Jorge A. Santiago-Blay and Joseph B. Lambert

With its fiery hue, amber has been valued for millennia for ornaments. Amber pieces, often hundreds of millions of years old, are even more treasured when they entomb plants, insects or other invertebrates or, more rarely, vertebrates such as amphibians and reptiles. Occasionally, the globules contain evidence of birds or mammals, such as a feather or fur. These inclusions were trapped in ancient sticky tree resin that then hardened and polymerized over the eons to become amber.

But whenever an object fetches a high price, fakes are soon to follow. Precious family heirloom jewelry from the early 1900s often turns out to be celluloid or Bakelite plastics. In Sir Arthur Conan Doyle's books, Sherlock Holmes investigated the fake amber industry in England in the 1800s. Even Chinese writers in the fifth century A.D. warned against forgeries made of eggs and fish oil.

More recent counterfeits—made of resins, plastics or both—can imitate amber's refractive index, specific gravity and ability to generate a static charge when rubbed. Fortunately, there does not yet seem to be one material that can duplicate all of amber's natural properties. Melted resin from contemporary trees, usually poured over some unfortunate creature, possesses amber's scent and its ability to retain warmth, but not its hardness. Modern polymers mimic amber's property of floating in salt water, but have not yet managed to give off the same piney scent when touched with a hot needle.

Determining a sample's botanical provenance is of interest to the scientist as well as the gemstone dealer and purchaser. Every day on the Internet commerce site eBay, unscrupulous sellers list numerous "amber" pieces that are obviously a sham to even a casual observer, yet unwary buyers still pay hundreds of dollars for these items. But if more skillful simulations were able to fool scientists, the consequences would be greater than just financial loss.

Amber is renowned as a preserver of ancient organisms, so paleobiologists study amber samples in an effort to learn more about Earth's past history. Amber specialists, ourselves included, are often asked to evaluate samples. If we were somehow erroneously to give our stamp of approval to a false piece of amber, any inclusion it contained might skew the direction of scientific investigation into that species' lineage. With such a serious outcome on the line, we began to wonder, could we be fooled?

Scientists can use more-modern tools and tests than a simple smell check to aid in their verification of a sample. Nuclear magnetic resonance (NMR) spectroscopy uses the magnetic properties of nuclei such as hydrogen or carbon to characterize different substances. The chemical environment of the nuclei influences their interaction with a magnetic field, as registered by what is called the chemical shift. The result is a spectrum of frequencies, reflecting the array of chemical species present in the material. This creates a sort of fingerprint for each compound in the sample.

But not all amber is the same. Deposits are found in several regions around the world and likely from more plant families than currently known from the fossil record. Therefore it's necessary to create NMR catalogs of many different kinds of amber. Besides helping to discern true from spurious samples, such a library has the added advantage of possibly indicating what kind of tree the amber may have come from, which could give us a better idea of the prehistoric landscape.

But to tell what tree amber came from, we may need to have something to compare it with as a starting point. Our approach has been to start with the exudates that modern plants ooze. There is no comprehensive library that relates NMR signatures to the taxonomy of modern plants that produce exudates. For the past 10 years, one of us (Santiago-Blay) has been amassing samples from all kinds of plants. We have now begun to outline the chemical picture of these gathered specimens of plant exudates.

Flowing Along

Resin is the only plant exudate that turns into amber, but it's not the only substance that trees ooze. Any organic material produced by plants, usually trees, which is extruded to the surface, can be labeled as a plant exudate. Besides resins, the most common are gums, gum resins, latexes and kinos.

Chemically, resins are made from complex arrangements of a five-carbon

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Figure 1. Polished to a warm luster, amber is a versatile medium for artwork. Its translucency works well for the flowing, carved lines of sculpture and finely crafted jewelry. The organic shape of the centerpiece of this necklace emphasizes amber's natural origins as ancient tree resin. Because it is highly valued, fake amber is also abundant. In the scientific realm, it is of utmost importance to ensure the botanical provenance of amber that preserves ancient organisms. Advanced chemical tests and a broad collection of modern plant resins may help provide the necessary evidence.


Figure 2. Which is real amber and which is fake? Some counterfeits are obvious to the naked eye; others look surprisingly real. Often mock amber contains large inclusions in perfect condition, something extremely rare in true amber. Vertebrates are particularly scarce in amber. Spurious examples made of plastics can mimic amber's hardness and its ability to float in salt water or refract light a certain way, but will not give off a piney scent when touched with a hot metal point. Fakes made of modern tree resins smell right but are too soft and often develop surface cracking. The scorpion-trapping sample on the left is real; the one on the right (containing a lizard) is counterfeit. (Images courtesy of Patrick R. Craig, *left*, and Doug Lundberg, *right*.)

molecule called isoprene. Thus they dissolve in many organic solvents such as chloroform or alcohols, but they are insoluble in water. When solid, resins tend to be amorphous, but they break in a shell-like, or conchoidal, fashion, because they are built up in layers as the goo flows down the side of a tree trunk.

Gums are composed of polysaccharides, which are complex molecular materials built up from simple sugars. Kinos are related to gums but contain polyphenols. Gum resins develop when carbohydrates from plant cell walls break down and are mixed with the building blocks of resins following damage to the structure that secretes resin. Latexes are based on isoprenes, like resins, but also contain complex mixtures of proteins, carbohydrates and phenolics; they are also produced by specialized structures called lacticifers. Latexes are not always opaque, and resins are not always transparent, so visual classification alone can often be inaccurate.

How does sticky plant resin becomes amber? The primary mechanism is cross-linking and polymerization of the isoprene units, which takes place under elevated temperatures and pressures over geologic time. These processes are also assisted by the chemical milieu in which the resin is deposited, such as the presence of acids and bases and maybe other compounds. Because resin is permeable, volatile materials originally present in the extruded resin, such as trapped gases, escape. Conversely, materials present close to the site of entombment, such as minerals, could get into the resin and give some of the hues found in rare ambers.

It is important to distinguish between hardening-to-touch and cross-linking and polymerization of the extruded resin, which forms the fossil solid we know as amber. A sticky resin can take from as little as a few weeks to a few


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Figure 3. Some of the most common plant exudates are resins, gums and latexes. Exudates often have distinctive physical properties, but looks can be deceiving—one tree exudes a white substance mistaken for latex but chemically shown to be a resin. Latex *(left)* is collected to make rubber in plantations in places such as Malaysia. Exudates from an Australian black wattle *(Acacia leiocalyx)* are likely gum but could be resin; chemical tests would be needed to determine which it is in this case *(middle)*. Resin from a *Boswellia* tree is the source of frankincense *(right)*.

years to harden. Early on in our studies of plant exudates, one of us (Santiago-Blay) collected resin from *Bucida buceras* L. (Combretaceae), a common urban ornamental tree in Puerto Rico. Unsuspecting of the true speed of hardening, Santiago-Blay approached the approximately 25-year-old tree with the nervous resignation that touching the exuded resin would lead to sticky fingers. He was surprised to find that the resins were hard. However, these solids are easily dissolved in ethanol and stronger solvents, such as xylene.

The second phase of fossilization, cross-linkage and polymerization, has been less studied. Some scientists suspect that it takes at least one million years for resin to become so polymerized and resistant to organics that it can be called "amber." However, some ambers are no match to stronger solvents. Once, by accident, one of us (Santiago-Blay) turned highly fractured chips of Arkansas amber (approximately 50 million years old, from the Eocene Period) into a mushy pulp. For some colleagues, this ability to dissolve amber can be quite useful, as they prefer to prepare microscopic biological inclusions preserved in amber by mounting the inclusion on a slide, as if it were modern material prepared on Canada balsam or modern human-made resins.

Solid resins that are neither ancient "amber" nor modern materials are called copal. They may date back hundreds to a few thousand years. Copals are usually solid throughout but are easily dissolved in ethanol. Last year, one of us (Santiago-Blay) received a scorpion entombed in "Dominican amber" housed in a central European collection. Having seen thousands of genuine amber pieces helps develop a search image for genuine amber, but it is by no means a guarantee. In this case, a simple test—a drop of 70-percent ethanol—rendered the specimen sticky, leaving fingerprints upon it, demonstrating that the specimen is not amber. Copals have been found in Madagascar, Colombia, the Dominican Republic and other countries.

Regrettably, copal is sometimes referred to as "semi-amber," undoubtedly a term introduced to lure naive buyers and to make additional profits from material that is, for now, far from the real thing. Exposed to the elements, copal is likely to fracture crazily across its surface and lose its value as jewelry.

Other plant exudates do harden as well but do not seem to last over extended geologic time. As far as we have examined, cycads, ancient plants that date at least from the origin of the dinosaurs (the Triassic Period, approximately 250 to 200 million years ago), produce exudates known as gums. The polysaccharide substance tends to have a spongy, watery consistency when oozed, making it vulnerable to rapid degradation by the weather and by organisms. However, as gums harden and cross-polymerize, they become nearly insoluble. The drying-up and hardening process can take as little as a few weeks, just like resins. Many other plants, such as members of the rose family (Rosaceae) and many legumes, produce gums.

In modern times, more than 160 families of vascular plants have been reported to produce exudates. Most of these plants are trees. Although most substances come from the shoot, significant root exudation is suspected for some ancient samples because of the large number of soil- and leaf-litter-dwelling creatures (for example, millipedes) trapped in amber.

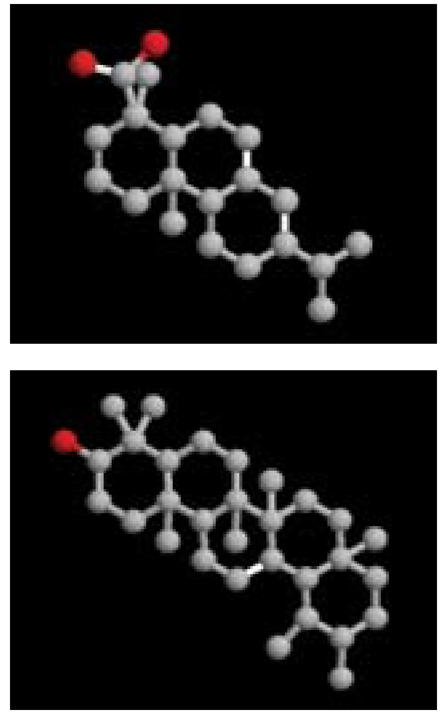

Figure 4. Resins are made from groups of five carbon atoms called isoprenes, which can be combined to form various structures. These compounds often can be used to identify the plant families that produced them. Oxygen is shown in red, carbon in gray, double bonds in white; hydrogen is omitted for clarity. The top resin, abietic acid, is in a class called diterpenes, whereas the bottom one is a triterpene called alpha-amyrin.

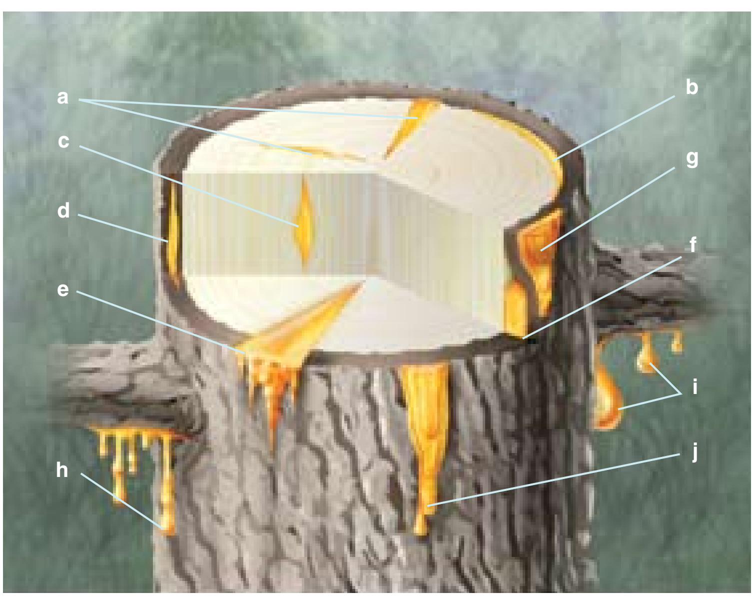

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Figure 5. Trees produce resin for numerous purposes. It fills cracks in wood *(a)* or between wood and bark *(b)*. It creates pockets within the stem *(c)* or the bark *(d)*. Resin acts as a bandage for wounds *(e)*, leaks into larger collections that cause bulges *(f)* or fills gaps in flat surfaces in layers that appear like growth rings *(g)*. When resin drips, it creates formations that look like icicles *(h)*, drops or tumescences *(i)* or stalactites *(j)*. Over the eons, as resin polymerizes to form amber, it can be clear or cloudy, or sometimes a combination of both *(above)*.

Figure 6. Amber does not have a crystal structure, but neither is it a single blob. This is because a chunk of resin, which will later become amber, does not usually form all at once but forms from several resin drips that accumulate into one larger accretion. The first wave of resin may stick to an insect like a glue trap *(a–b)*; subsequent flows will encase it *(c–d)*. Lines from such successive drops of resin are visible as streaks in this amber piece that trapped a hymenopteran insect *(above)*. Amber glows under ultraviolet light, which makes its numerous flowing layer boundaries readily visible. Fakes rarely have this property.

Different types of exudates are produced by different types of botanical tissues. For instance, resins have been reported to be produced by either epithelial-derived "blisters" or vascular-derived canals, whereas gums come from ground-tissue-derived cells and latexes are generated by phloem-derived laticifers.

Figure 7. Western pine beetles bore tubes into the living part of a tree's stem. In response, trees produce resin that fills the tube. The resin can trap the offending insect, one mechanism by which ancient organisms became entombed in amber. (Photograph by Clyde D. Wilson, from *Pests of the Native California Conifers,* by David L. Wood, Thomas W. Koerber, Robert F. Scharpf and Andrew J Storer, courtesy of University of California Press.)

It appears that exudation is a natural mechanism plants have for healing wounds caused by organisms or by the environment, such as lightning or fire. Extreme environmental conditions also seem to affect exudate production. For instance, some gum producers increase their levels when they endure a dry climate. But other uses are possible. Observations by Francis Huber and Carol Hotton from the National Museum of Natural History have led them to infer that a possible exudate may have served as a mechanism for adherence of spores in ancient plants from the Devonian Era (417 million to 354 million years ago).

Exudation is widespread in space and time as well as in taxonomy. There is evidence of plant exudates produced as far north as Axel Heilberg Island (Canadian Arctic, 80 degrees North) and as far south as New Zealand (40 to 45 degrees South). Exudates have been produced at least since the Carboniferous (circa 300 million years ago) by the Holarctic medullosan seed ferns, *Myeloxylon.* The microscopic remnants, known as "resin rodlets," have a unique chemical composition. Nobody knows whether this observation represents ancient reality well or is the product of millions of years of chemical change. Recently, relatively young amberiferous deposits have been discovered in Australia.

Amber forests were often associated with plant tracts in low elevations near water. In fact, numerous old illustrations portray amber forests in association with aquatic communities, frequently at sea level. We once worked on a piece of amber from Chiapas, located in southeastern Mexico. Numerous geological studies confirm that this is an active geological region, supporting the idea that the amber forest, at least in Chiapas, made out of the legume *Hymenaea,* was close to sea level and that geological activity in the last 15 million years has lifted this forest to its current elevation, some 1,000 meters above sea level. The amber had inclusions that could be a tubeworm, a fingernail clam, the base of a barnacle and quartz sand. Other studies have reported coastal organisms in amber.

The Avid Collector

Since amberiferous forests were often associated with low-lying areas, amber deposits were often washed into rivers and subsequently the sea. This may be a reason why the Baltic coast has always been a major source of amber. Some families in that region have made their living for generations by collecting amber from the sea. One of us (Santiago-Blay) has joined their ranks as a collector—not of amber but of fresh plant exudates. Since 2001 Santiago-Blay has visited about 50 botanical gardens in the United States and has taken advantage of their assemblages of plant species from around the globe to collect samples from more than 800 exudate-producing species.

Often inquisitive visitors ask Santiago-Blay why he is so attentively close to a tree trunk while holding a knife and a tiny zipper-closure baggie. As plant exudates are naturally emitted by the plant, they can be simply scraped from the trunk surface without any damage to the tree.

Santiago-Blay often collects samples with students and other volunteers, who enjoy the "thrill of the hunt" in locating species that produce an exudate. Although collecting takes place at all times of the year and in all weather conditions, roaming around these large, beautiful gardens is a most peaceful and rewarding experience. Indeed, one of the most relaxing times to collect is

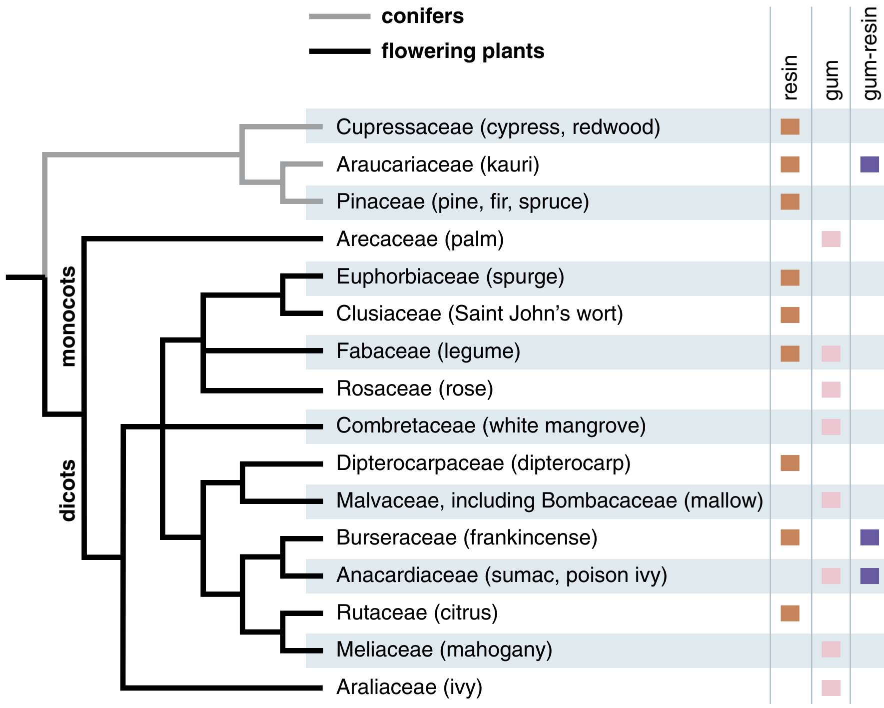

Figure 8. A taxonomy diagram shows some families of plants known to produce exudates. Names in parentheses indicate a common plant in each family. Both flowering plants (divided by seed type into monocots and dicots) and conifers are widely represented. Families of plants produce resins, gums, gum resins or combinations of these exudates. Recent unpublished research in our laboratories has revealed that *Prosopis juliflora* of the Fabaceae family, and *Guaiacum officinale* and *Guaiacum sanctum* of the Zygophyllaceae family, produce a new class of exudates not included in this figure.

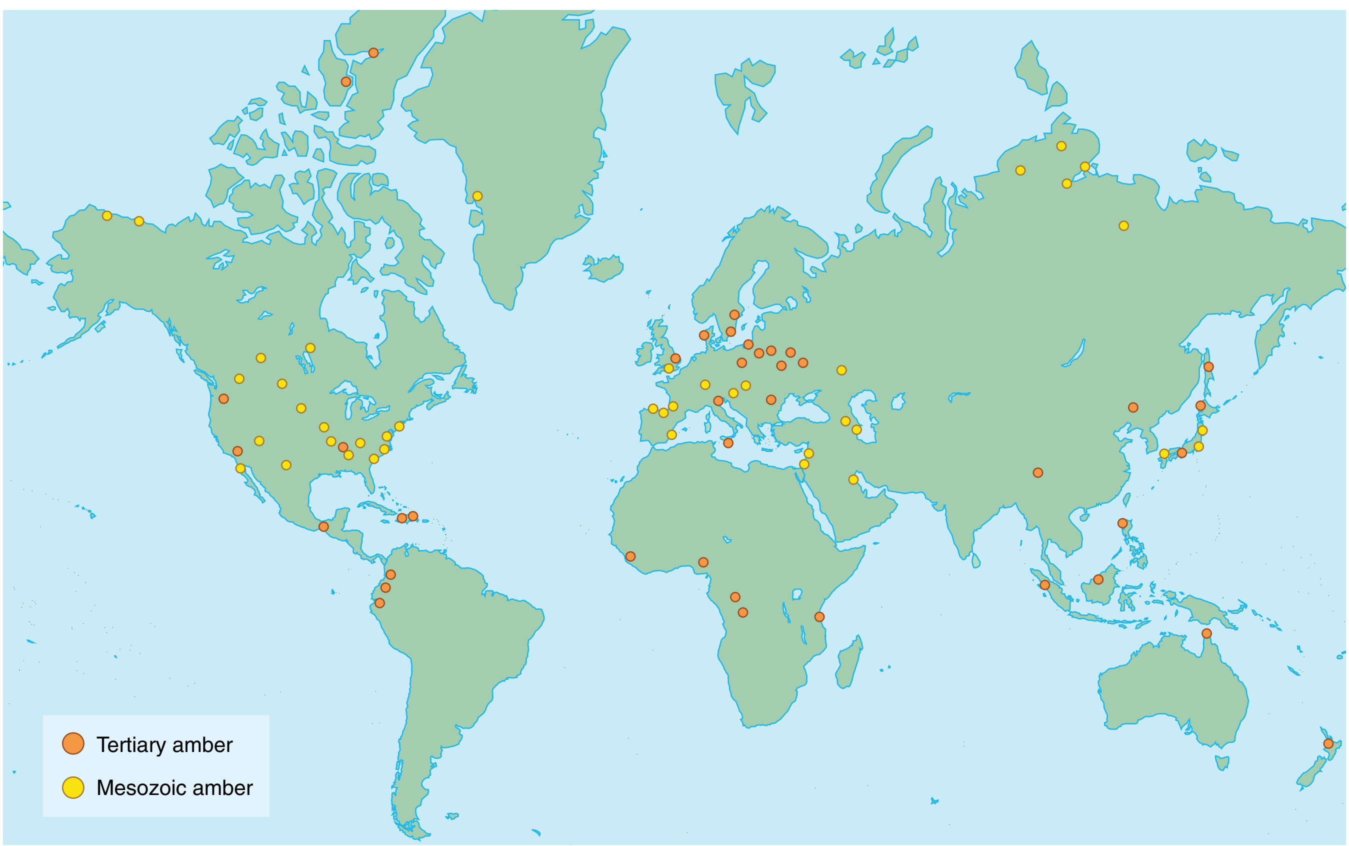

Figure 9. Major deposits of amber and copal, dated from different time periods, have been found worldwide. Numerous other sites are also known, and more are being regularly discovered.

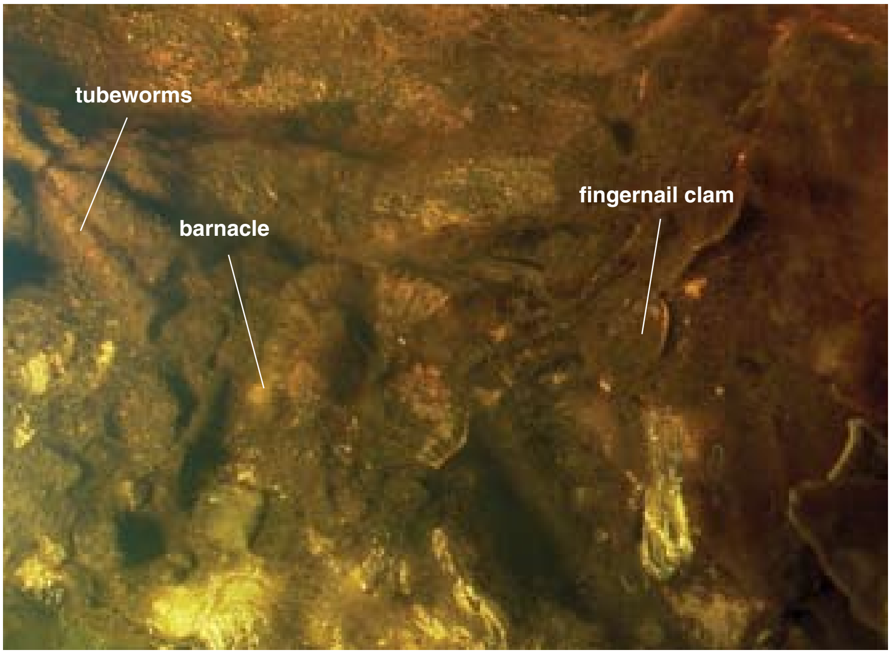

Figure 10. A close-up of a piece of Chiapan amber shows what appears to be tubeworms, the base of a barnacle and a fingernail clam. Other amber pieces have been found containing quartz sand. These provide evidence that amber may have formed close to a marine environment. Ancient trees that were the source of this amber may have dripped resin into the water, where it settled and trapped marine specimens before hardening into a solid. (Photograph courtesy of Patrick R. Craig.)

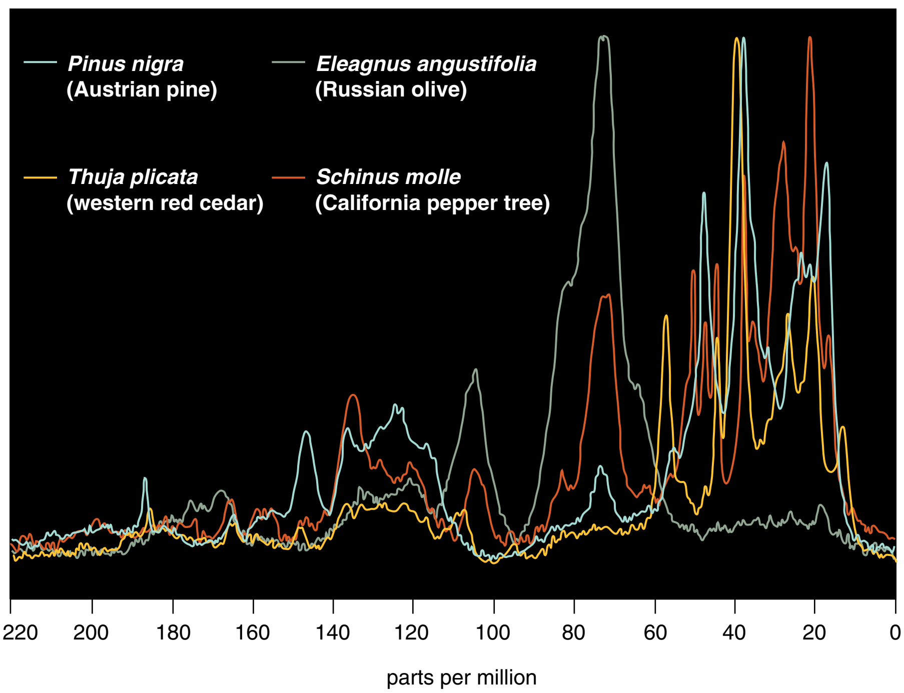

Figure 11. Carbon-13 nuclear magnetic-resonance spectroscopy of exudates from different species shows that not only are gums, gum resins and resins distinguishable, but also that the patterns of peaks from the same type of exudate vary significantly by the species that produced the substance. This result indicates that the individualized chemistry of exudates often can be used to identify at least the family of plant that produced them, a technique that could be extended to determine the types of ancient trees that produced amber. Resins produce a series of sharp peaks at the right end of the spectrum, whereas gums produce prominent peaks toward the middle of the spectrum; gum resins produce both of these peak sets. Two spectra shown are from different types of resins, from a pine and a cedar. Other spectra show a gum from the Russian olive, *Eleagnus angustifolia*, and a gum resin from the California pepper tree, *Schinus molle*.

following a snowfall, when the gardens are silent and deserted.

Every garden is different, and thus every collecting experience has its own story. When arranging permission to collect at one garden in Ohio, Santiago-Blay was invited to stay in the unused custodian's house on the grounds during the visit. He accepted gladly, expecting no more than a cot to sleep on. To his surprise, the "house" turned out to be an incredible mansion, at his disposal for the entire visit. At this garden he collected 73 samples, some of which were from species not found in any other garden to date. At the other end of the spectrum, while visiting central Oregon, accommodations were so scarce that Santiago-Blay was forced to spend the night on a hard asphalt parking lot, all the while fearing the bears known to be in the vicinity.

But the true reward of collecting these samples is when we perform a chemical analysis on a new specimen, using NMR spectroscopy. Analyses, performed in collaboration with Yuyang Wu and Michael A. Kozminski of Northwestern University, are carried out on the carbon-13 (^{13}C) or hydrogen (^{1}H) nuclei that are present naturally in organic molecules. With ^{13}C NMR, the resulting spectrum represents the entire sample rather than a limited component as some other techniques do. We use ^{13}C NMR spectroscopy for analysis of solids and ^{1}H NMR spectroscopy for analysis of liquids (samples dissolved in a solvent). For ^{13}C spectra we require about 100 milligrams, and for ^{1}H spectra less than 20 milligrams, of a substance. Samples may be recovered, so the procedure is nondestructive.

Because the chemical nature of exudates may vary with the species of plant, the characterization of modern exudates by different physicochemical techniques can establish the likely botanical origin of ancient resins. The analyses of ^{13}C NMR spectra from several plant genera as well as our analyses using ^{1}H NMR have disclosed wide variation in spectral signatures between, not within, plant exudates. On the basis of these finds, it appears that NMR provides a new diagnostic test for identification of ancient exudates and assessment of taxonomic relationships between plants.

Although in many cases the structure of the traditional botanical families has been confirmed by ^{13}C NMR, this has not always been the case. For instance, morphological and molecular-genetic evidence garnered by numerous research groups during approximately the last 15 to 20 years have demonstrated the unity of the cypresses (family Cupressaceae) and the redwoods (formerly family Taxodiaceae). Interestingly, those two groups of plants have almost indistinguishable NMR profiles supporting the one-family placement of the Cupressaceae + Taxodiaceae.

On the other hand, ^{1}H NMR is able to distinguish every one of the eight genera tested belonging to the pine family (Pinaceae). In addition, our NMR studies of Burmese amber (approximately 100 million years old, from the Cretaceous Period) and their inclusions have confirmed the presence of plants in Araucariaceae, a family of conifers that includes the monkey puzzle tree and the "living fossil" Wollemi pine. Macrofossils of this botanical family have already been found for Burmese amber.

The botanical identity of Tertiary amber samples from North Carolina has confirmed previous paleobotanical finds of other investigators. Our recent NMR data indicate that legumes and pines oozed resins, which eventually became amber. Fossils of these types of plants have been found in the region and from the time period of our amber samples, reinforcing our results. Although fossil cypresses have also been found in North Carolina amber, we have not yet discovered those in our NMR analyses.

Something Old, Something New

Years of experience allow students of amber to develop a good gestalt feeling for the real thing. However, we are regularly reminded that there is no substitute for performing rigorous tests to demonstrate the authenticity of a sample. Recently, a specimen of Chiapan amber that appeared to contain small eggs was brought to our attention. Although the specimen fizzled when acid was added, suggesting the presence of carbonates, such as is found in eggs, a closer inspection made us believe that the egglike structures may be carbonate mud shaped into an ovoid. This only underscores the need for chemically sophisticated tests to verify the true nature of amber samples, rather than trusting one's expertise to tell the difference between the genuine and the imitations.

Our hope is that once we have analyzed a sufficient number of plant-exudate samples, we will be able to develop a comprehensive classification scheme for resins and other exudates that relates chemical structure directly to plant taxonomy. Once we reach that point, we may begin to provide more-concrete data on the families of trees that produced these ancient gems. We may be able to rediscover and reconstruct the ancient amberiferous forests of the world more completely, based on just these small cast-offs that they have left behind for us from eons past.

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