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The range of life in amber: significance and implications in DNA studies

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Abstract. A survey of the major fossiliferous amber deposits is provided, including ages and various categories of life forms reported from each. The frequence of occurrence of the major groups of plants and animals in these amber deposits is also given. Thus far, DNA from four insect and one plant species has been extracted from amber fossils. In the case of the stingless bee in Dominican amber, evidence of reproducibility is provided, since two independent laboratories isolated DNA from six or more different specimens of the same insect.
Amber sources for DNA studies are listed together with their advantages and disadvantages. The important points are the availability of desired pieces, the proper identification of the fossil, verification of the amber deposit, the cost involved, and the feasibility of causing damage to the specimen. The availability of several types of amber (Mexican, Dominican, Baltic, Chinese, Canadian, Siberian and Lebanese) at four major sources (academic collections, commercial dealers, private collections and amber mines) is discussed. The scientific implications of obtaining DNA from amber inclusions are presented.
Key words. Life forms in amber; amber sources; DNA studies with amber organisms.

Diversity of life in amber

Amber inclusions have been studied for over 200 years, most of the early reports dealing with fossils in the famous Baltic amber deposits. The recent reports on the recovery of DNA from amber inclusions have added a new dimension to amber research, namely that of molecular paleontology. Thus amber is no longer only of interest to the traditional, classical scientists, of whom few are left today, but also to the new generation of molecular systematists and ultimately to those in the field of genetic engineering.
Major fossiliferous amber deposits (see table 1) occur throughout the world. Their location is correlated with the past occurrence of forests of certain species of trees which produced the resin which fossilized into amber. Most of the amber listed in table 1 was formed by either members of the coniferous family Araucariaceae (especially the older Cretaceous material) or members of the angiosperm family Leguminoseae (Mexican and Dominican amber).
These amber deposits span a range of over 100 Myr and contain a variety of organisms, including microorganisms, invertebrates, vertebrates and plants. A systematic listing of the major groups of life forms in amber together with their frequency of occurrence is shown in table 2. Microorganisms occur in all of the major fossiliferous amber deposits with fungi being seen frequently (fig. 1). This is because saprophytic fungi often occur on the bodies of poorly preserved insects in amber. It is quite possible that bacteria are just as common, but simply have escaped notice because of their size. Mosses and liverworts occur in amber from most deposits since these plants frequently grew on the bark of trees in humid climates and could easily be covered by resin flows. Evidence of gymnosperms is rare in amber in general, and in fact is unreported in all but Baltic amber. From this latter source are reports of pines, firs, larches, sequoias, cypresses and junipers[18]. Again, while remains of angiosperms occur in all of the amber deposits listed in table 1, very few of these plants

Table 1. Major fossiliferous amber deposits (with over 1000 biological inclusions reported)

Amber type	Location	Age (Myr)	Life forms
Mexican	Chiapas, Mexico	22–26	Microorganisms, invertebrates, vertebrates, plants
Dominican	Dominican Republic	25–40	Microorganisms, invertebrates, vertebrates, plants
Baltic	Northern European countries bordering on the Baltic and North Seas	40	Microorganisms, invertebrates, vertebrates, plants
Chinese	Fu Shun Province, China	40–50	Microorganisms, invertebrates, plants
Canadian	Manitoba and Alberta, Canada	70–80	Microorganisms, invertebrates, plants
Siberian	Taimyr Peninsula	75–115	Microorganisms, invertebrates, vertebrates, plants
Lebanese	Southern Lebanon	120–135	Microorganisms, invertebrates, plants

Table 2. Range of life forms in amber

Major groups	Frequency of occurrence*
Bacteria	Occasional
Protozoa	Rare
Fungi	Common
Mosses and Liverworts	Rare
Gymnosperms	Rare
Angiosperms	Occasional
Nematodes	Rare
Annelides	Rare
Molluscs	Rare
Crustaceans	Occasional
Myriapodes	Occasional
Insects	Common
Spiders	Common
Scorpions	Rare
Mites	Occasional
Frogs	Rare
Lizards	Rare
Birds (feathers)	Rare
Mammals (hair, epidermal cells)	Rare

*Common, occasional or rare based on published reports and personal observations.

Figure 1. A fungus growing on an insect in amber from the Dominican Republic. Many saprophytic and some parasitic fungi occur on a range of arthropods in amber.

Figure 2. A wood louse in amber from the Dominican Republic. These isopods are the most common type of crustaceans found in amber.

have been identified except those in Baltic amber. From this source are descriptions of palms, grasses, maples, holly, elderberry, heather, oak, beech, geranium, magnolia, smart weed and buckthorn, to name about half[18]. As soon as sequences of modern plants are better known, then DNA studies can be involved to aid in the identification of plant families and genera. Plant identifications assist greatly in the reconstruction of the original landscape and climate during the period of resin production. Nematodes, molluscs and annelids are rare because, aside from a few forms that occur in rotting bark and some insect parasites in the case of nematodes, most representatives of these groups are in or on the surface of the soil. Of the crustaceans, wood lice (Isopoda), which were probably living under the bark of the resin-producing trees, are most common (fig. 2). The amphipods, to which the beach hoppers belong, occur on the forest floor and are rarely seen in amber. Of the myriapods, millipedes are the most commonly encountered group in amber (fig. 3). These plant and detritus feeders are known to occur under tree bark.

Insects are certainly the most common arthropods found in amber. In Dominican amber alone, there are over 235 insect families present[18]. This number is increased significantly when combined with insects reported from other amber deposits, especially representatives of now extinct families from Cretaceous deposits (fig. 4). It is thus understandable that spiders are also common, since the great majority prey on insects and many forms live under tree bark. Other arachnids such as scorpions and their relatives are rare and are valued at thousands of dollars. Mites are small and often escape notice – thus they are usually more abundant than presumed (fig. 5).

All vertebrates in amber are rare and difficult to acquire. Lizards and frogs are the only vertebrates that are represented in their entirety in amber, although more often than not they have portions of their bodies missing (fig. 6). Birds and mammals have been represented in amber only by feathers (fig. 7) and hair, respectively. Forensic methods have been used to identify hair[19] but molecular studies could provide a more precise identification.

DNA extraction from amber inclusions

The organisms in amber from which DNA has been extracted are shown in table 3. All of these are extinct at the specific or generic level. Reproducibility has been demonstrated in the case of the fungus gnats (*Orfelia* spp.) and the stingless bee, *Proplebeia dominicana*, both from Dominican amber. Regarding the bee, it should be noted that DNA was successfully extracted in two separate laboratories from separate specimens, and in one laboratory, specimens from five pieces of amber all were positive for DNA.

Figure 3. A milliped in amber from the Dominican Republic. These plant and detritus feeders occur in debris, sometimes under tree bark.

Figure 4. A member of the extinct family Serphitidae (Hymenoptera) in Canadian amber (specimen collected by T. Pike). This family is one which seems to have become extinct at the K-T Boundary.

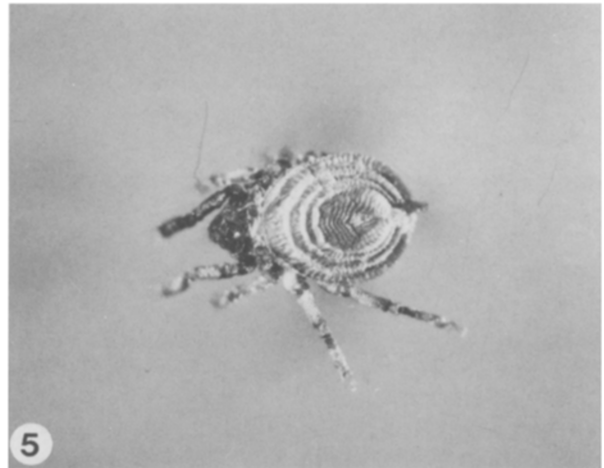

Figure 5. A mite in amber from the Dominican Republic. Because of their small size, they are frequently overlooked.

Figure 6. A frog in amber from the Dominican Republic. Note that portions of the two right legs are missing.

The sources of amber for DNA studies can be grouped into four major categories (table 4): namely, academic collections (museums, institutes and universities), commercial dealers, private collections and specific amber mines. The various advantages and disadvantages associated with each of the above sources are outlined in table 4.

Table 3. Organisms in amber from which DNA has been extracted (in chronological order)

Type of material	Identification	Number of specimens	Amber source and age (Myr)	DNA Stages			
				Extraction	Amplification	Sequencing	Reference
Insect	*Proplebeia dominicana* (Apiae: Hymenoptera)	5	Dominican amber (25–40)	+	+	+	Cano et al.[3,4]
Insect	*Orfelia* (sensu latu) spp. (Mycetophilidae: Diptera)	4	Dominican amber (25–40)	+	+		Poinar, H.N. unpubl. data (Dec., 1992)
Insect	*Mastotermes electrodominicus* (Mastotermidae: Isoptera)	1	Dominican amber (25–40)	+	+	+	DeSalle et al.[6]
Insect	*Proplebeia dominicana* (Apidae: Hymenoptera)	1	Dominican amber (25–40)	+	–	+	Hoelzel[10]
Insect	*Libanorhinus succinus* (Curculionoidea: Coleoptera)	1	Lebanese amber (120–135)	+	+	+	Cano et al.[5]
Plant	*Hymenaea protera* (Leguminoseae: Angiospermae)	1	Dominican amber (25–40)	+	+	+	Poinar et al.[21]

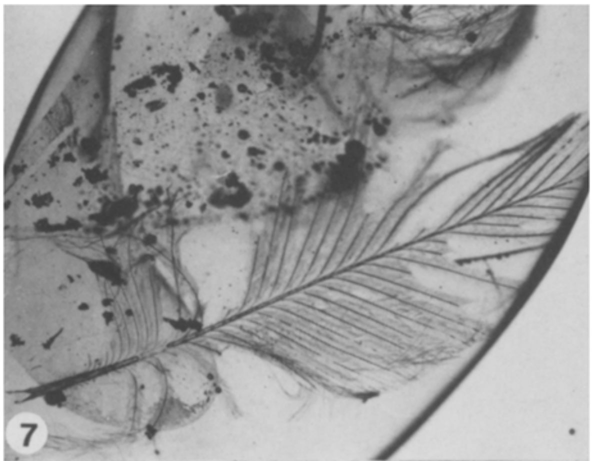

Figure 7. A feather in amber from the Dominican Republic. Feathers are the only remains of birds in amber.

Figure 8. The weevil, *Libanorhinus succinus*, in amber from Lebanon. In order to preserve the specimen after DNA extraction of the internal tissues, the pieces of amber were glued back together and the composite piece embedded in plastic for stabilization and protection from handling and the atmosphere.

Recently, a plea has been made to avoid the often destructive methods employed to obtain tissue from amber insects for DNA extractions[13]. Nobody wants to destroy a fossil specimen if they can avoid it. First, it is important that the specimen be properly identified before DNA extractions are initiated. In all cases regarding the organisms listed in table 3, the specimens had either been previously identified or they were described at the time of the extraction. Unfortunately, many of the trained systematists have retired or are close to retirement and many of their positions are not being filled. It is startling to realize that while everybody is concerned about biodiversity and the rapid disappearance of plant and animal species on the planet today, the scientists that have been trained to identify and describe these specimens are themselves becoming an endangered species. Yet, it is their taxonomic studies that form the basis which population biologists use to evaluate the effects of various environmental disturbances (mostly caused by human activities) on present day populations of animals and plants. Perhaps the new generation of biologists feels that it will soon be possible simply to remove a bit of DNA from an organism in question, obtain a couple of sequences, enter them into a genebank and obtain a name for the creature. Will new species then be characterized only by their sequences? This is something to be decided in the future and does not warrant the dismissal of classical biological taxonomists at this time.

Obtaining amber specimens from institutions for DNA analysis has the advantages that the specimens are usually either identified or can be identified. They are easily available at no or little cost (at present) and the source is usually verified (in some museums, the pieces may be in open boxes and mixing of specimens could have occurred). Rare and type specimens would probably not be available and there would be regulations regarding how much damage would be permitted on each specimen. Several methods could be employed to minimize the damage to rare specimens from which DNA is to be extracted. If the specimen is cracked open, it can be glued back together after the tissue has been removed. Such was done with the weevil *Libanorhinus succinus* in Lebanese amber. The piece of amber containing the weevil was very fragile and actually broke into three portions. All three were glued back together and the

Table 4. Amber sources for DNA studies

Source	Advantages	Disadvantages
Academic collections (museums, institutions, etc.)	– availability – identification usually provided – cost minimum – source usually verified	– restrictions regarding damage to specimens
Commercial dealers	– availability – can damage specimen	– identification usually needed or confirmed – source may need verification – payment required
Private collections	– availability – can damage specimen	– identification may be needed or confirmed – source may need verification
Amber mines	– can verify source – can damage specimen	– travel expenses – dangers (cave-ins) – finds unpredictable – permission usually needed – identification needed

entire piece was then embedded in clear plastic for protection (fig. 8). Although the abdomen has had the tissue removed, many of the external morphological characters are still present. Another technique is to drill a small hole through the adjacent amber and through the cuticle of the specimen[20]. A small flexible tube attached to a syringe is inserted through the hole, and some of the dry tissue is withdrawn and placed directly into the extracting medium. Of course the amber piece, as well as all the equipment, have to be previously sterilized.

Amber specimens from other sources normally require identification and in some cases verification regarding the original locality. When you purchase a specimen, you many encounter a forgery. Forgeries could be the subject of a separate paper. Suffice to say that there are relatively easy ways to detect most forgeries[15] and they all contain present day life forms.

Travelling to an amber-producing area and chipping out your own piece of amber from rock strata in a mine is an extreme way of being certain about the source of your amber specimen. This was what our group did when we wanted authentic amber from various mines in the Dominican Republic for nuclear magnetic resonance samples. However, you may have to work for several hours before finding some fossiliferous amber, and then the fossils you find are unpredictable. Travel expenses and various dangers (cave-ins and armed guards) make this method generally unattractive except for the adventure hunters.

Certain types of amber are much more available than others (see table 5). Up until the last few years, amber from the Dominican Republic was the common type found in shops in North America. It was dug in various mines and the demand for jewelry and fossils kept a constant supply entering the United States. Now, independence in the Baltic States and eastern Europe has removed previous restrictions that surrounded the exportation of Baltic amber and the fossiliferous material entering the States has increased significantly. Amber from Mexico is not as abundant as that from the Dominican Republic and most is not exported, being sold to tourists in Mexico City. As a result, there is a flourishing trade of plastic fakes coming from Mexico. Older amber from Cretaceous sources is mostly available from small museum collections which in some cases may not yet have been inventoried and thus the contents largely remain unknown (e.g. Canadian amber). Long distances, relatively unknown localities and political unrest discourages most scientists from collecting their own amber. Aside from those sources listed in table 5, there are a number of smaller fossiliferous amber deposits scattered throughout the world[18]. Again, adventurous explorers may search these out or actually discover some new sites. If this is done, then aside from all of the above mentioned requirements, the researcher would also have to determine the age of the amber.

Preservative qualities of resin and amber

Now that DNA has been extracted from amber inclusions, a few words should be said about the components in the resin which actually fix the tissues and DNA for such a long time. That the tissues and cells are fixed has been demonstrated with electron micrographs of a Baltic amber midge showing cell organelles including nuclei and mitochondria[16]. Just what components of the resin are responsible for this remarkable degree of preservation? We speculated that the sugars in the resin withdrew the moisture from the original tissue and thus initiated the process of inert dehydration[16,17].

Although there is much discussion in the literature regarding resin quality and quantity, very few studies have been done on the actual components in the resin. Most of the studies that have been reported on this topic were done on the trunk resin of *Agathis australis*, a member of the Araucariaceae that grows in northern New Zealand. It was mainly because the resin had a commercial use at one time (still today in part) that these studies were conducted. The results of these investigations, summarized by Cambie[2], are presented in table 6. First, the sugars glucose, galactose and arabinose are present as well as various acids, alcohols, an ether, ester and of course various terpenes. One of the oxygenated derivatives of terpene hydrocarbons are aldehydes, which may serve as a fixative of the embedded tissue. Hydrogenated derivatives such as methane may also have similar effects. Aside from inert dehydration which stabilizes the proteins, the acids theoretically could create a problem regarding the stability of DNA. It will be interesting to explore whether the exterior

Table 5. Availability of different types of amber

Amber type	Academic collections	Commercial dealers	Private collections	Amber mines
Mexican	+	+	+	+
Dominican	+	+	+	+
Baltic	+	+	+	−
Chinese	+	−	−	−
Canadian	+	−	?	+
Siberian	+	−	−	?
Lebanese	+	−	+	−

Table 6. Components in the trunk resin of *Agathis australis* (Araucariaceae) (Modified from Cambie[2])

Carbohydrates	Alcohols	Acids	Ethers	Esters	Terpenes
glucose	fenchyl	agathic	glycol ether	agathic acid-monomethyl ester	α-pinene
galactose	cis-communol	abietic			limonene
arabinose	trans-communol	cis-communic			dipentene
		trans-communic			

barriers of insects and plants (cuticle) may serve to protect the DNA from the initial actions of resin acids. Clearly more studies are necessary on components found in resins and amber and their effect on plant and animal cells. The studies on *Agathis* resin are appropriate since the plants responsible for the Baltic amber and the amber occurring in Canada and Lebanon are also considered to be derived from extinct representatives of the same family and possibly genus[1,11].

The ability of DNA to survive in amber inclusions for millions of years contradicts traditional views on earlier models of DNA instability, showing decay after thousands of years[12]. However, none of these models were based on DNA in fossil material, especially in amber, and therefore they have no direct relationship to the present studies. It is now obvious from the variety of ancient sources from which DNA has been extracted that each environment has its own set of biological and physical parameters that will collectively affect the rate of DNA decay.

Evolutionary significance

The extraction, sequencing and comparison of DNA from fossils ranging from 22 to 139 million years of age provides great potential for a variety of studies. First of all, plant and vertebrate remains too fragmentary to be identified by morphological methods could possibly be identified from molecular sequences. Second, by comparing the sequences of these ancient fossils with sequences of their closest extant descendants, we can learn much about evolutionary changes, mutation rates and molecular clocks. Phylogenetic data from amber fossils can be combined with subfossil or even historical species which have been displaced or become extinct, thus providing various time points for constructing a phylogenetic history of plant and animal genera. Paleomolecular data can also be used to solve biogeographical problems which require precise comparisons with extant populations[20]. In certain cases, such data could even be used to distinguish between past dispersal and vicariant events. Thus amber fossils can have a significant role in the emerging field of molecular evolution and paleontology.

Future implications

There are many additional types of studies that could be undertaken with amber inclusions, which involve novel and generally inaccessible sources of ancient DNA. What we have in amber is essentially a micro-zoo and botanical garden of the past. More importantly, in the Mexican and Dominican ambers are representatives of past neotropical rainforests, similar to those being destroyed today in the Amazon. Our present tropical rainforests have an ecosystem that harbor the greatest diversity of life on earth. Although the tropical rainforests cover only 7% of the earth's land surface, they contain more than half the species of the world's animals and plants. It is considered that these forests will disappear within the next century, eliminating hundreds of thousands of animals and plants[23].

Just consider all the genetic diversity that will disappear with the loss of these species. Genetic diversity is essential to the longterm survival and adaptability of all life. Most species consist of multiple breeding populations that contain a high amount of genetic diversity. This genetic variation is reduced when populations of a species are destroyed. When a plant variation is reduced to a few populations or is genetically selected by man, much of its genetic diversity is lost: often forever, according to Wilson[23]. As pointed out by Ehrlich[8], genetically diverse populations are vital to the survival of a species under changing environmental conditions.

And the genetic diversity within species is declining rapidly over the earth's surface. An example of what can happen with reduced genetic diversity was demonstrated some years back with the program called the Green Revolution[14]. Scientists selected high yielding crops which were grown in third world countries. At first the yields were so high, that the farmers threw aside their old seed and everyone planted the new strains. However, while the yield was high, the adaptive, self-sustaining capacity of these strains was sacrificed. They were greatly dependent on high levels of fertilizer and pesticides to survive. When the care they required became too much, the plants failed. They didn't have the genetic diversity to sustain thermselves. In the end, the farmers scrambled around to find the old seed they had originally discarded.

We have in amber a genebank stored in the remains of plants whose DNA is potentially retrievable. Many are rainforest plants. The fossil record of rainforest plants (and animals) is very poor[22] since the organisms decay rapidly after death and the fossilization potential is almost nil. As warned by Anne and Paul Ehrlich[7], the expanding human population is leading to the extermination of wild plant species and populations around the world. Some of the plants being lost are wild relatives of domestic crops which are potential sources of valuable genetic information that could be incorporated into the crops by breeding programs.

In amber are representatives of the two most important food crop groups in the world today – legumes and grasses. Genetic material from these fossils could be inserted into contemporary plants to re-establish genetic diversity – possibly resulting in an increase in yield and a greater tolerance to climatic fluctuations, pest and diseases.

According to Farnsworth[9] approximately 119 chemicals from higher plants are used in medicine by almost 3.5 to 4 billion people throughout the world. In 1985, the American consumer spent in excess of $8 billion on drugs whose active ingredient was extracted from higher plants. Today pharmaceutical companies are reluctant to initiate new programs involving plant drugs for the following reasons:

1) lack of patent protection,
2) difficulty in obtaining a continuous supply of plants, and
3) variation from one lot of plants to another.

Since plant remains in amber are not naturally occurring today, their DNA sequences could be patentable. Clearly it will not be long before plant sequences controlling the production of medicinally active ingredients will be cloned and the need for a continuous supply of plants or the problem of variation between batches of plants would be resolved. This means that plants in amber could represent a source of bioactive plant products in the future.

Also related to medicine are discoveries that could be made in relation to blood-feeding arthropods in amber. In amber are mosquitoes (Culicidae) which transmit encephalitis viruses, malaria plasmodia, and filarial nematodes; horseflies (Tabanidae) which carry tularemia, anthrax *Bacillus*, protozoa and worms; biting midges (Ceratopogonidae) that are vectors of filarial worms and viruses of birds and animals; sandflies (Psychodidae) which carry rickettsia, virus and several types of protozoa; blackflies which transmit protozoa and filarial nematodes and fleas that are responsible for vectoring pathogens causing plague and murine typhus. Aside from insects, there are also parasitic mites and ticks in amber – the latter of course well known for their association with organisms causing encephalitis, hemorrhagic fever, relapsing fever, typhus and of course Lyme disease.

It might be possible to find the early evolutionary stages of these parasites in blood-sucking arthropods in amber. An examination of critical sequences should enable us to better trace the origin of these pathogens. On the other hand, it may be possible to discover primitive basic sequences that could be used for the production of vaccines. At this stage, these organisms might not yet have developed the mutability we now experience that allows them to change their surface and confuse the host's immune system.

These are just a few of the possibilities that could be explored when ancient sequences from fossils are found and expressed. Amber provides the opportunity to examine DNA from animal and plant inclusions millions of years old and to obtain certain secrets from them that have been locked away for eons.

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