spectra. Large differences are seen in other ambers (burmite, schraufite, walchowite), including appearance of ketonic resonance, loss of *exo*-methylene resonances, and increase of acid and ionized acid carbonyls at the expense of esters. We hope that these newly established constitutional differences can be exploited not only as a diagnostic for geographic sources but also as an aid in the identification of the paleobotanical origin of amber through comparison with spectra of modern resins.

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Spiroplasmas: Diversity of Arthropod Reservoirs and Host-Parasite Relationships

Abstract. *Spiroplasmas were found in 11 new insect hosts representing four orders: Hymenoptera, Hemiptera, Diptera, and Coleoptera. Three of the new spiroplasmas were serologically distinct from all existing groups or subgroups. A spiroplasma that infects digestive tracts of Colorado potato beetles may be transmitted to uninfected insects that feed on contaminated plants. This simple type of spiroplasma-insect relationship may explain a growing list of isolations of spiroplasmas and other wall-less prokaryotes from plant surfaces.*

Although spiroplasmas were first envisioned (*1*) in 1961 and gained recognition (*2*) as a distinct microbial group in 1973, until 1975 only three such organisms were known. Two of these spiroplasmas, the agent of citrus stubborn disease (*3*) and the agent of corn stunt disease (*4*), cause economically important plant diseases that are transmitted by leafhoppers (*5*). A third spiroplasma causes the elimination of male progeny from broods of infected females of certain Neotropical species of *Drosophila* and is called the sex ratio organism (*1, 6*). The suckling mouse cataract spiroplasmas (*Spiroplasma mirum*) (*7*), although originally isolated from rabbit ticks in 1964 (*8*) and long thought to be a virus, was eventually recognized as a spiroplasma (*9, 10*). The discovery of a spiroplasma in the honey bee (*11*) first focused attention on flowers and plant surfaces as possible sites of insect-to-insect transmission because the seasonal occurrence of the organism in the hemolymph of bees was synchronized with the spring nectar flow. Flower and plant surfaces have since proved to be rich sources of spiroplasmas and other wall-less prokaryotes (*12, 13*). On the basis of my findings, it seems likely that many of these organisms are inhabitants of the intestines of insects and are transmitted from insect to insect by contamination of plant surfaces.

The insects were collected at the USDA Agricultural Research Center in Beltsville, Maryland (*14*). Honey bees used in these experiments were from colonies maintained at the Bioenvironmental Bee Laboratory. Many insect species were examined for spiroplasmas (*15*) (Table 1).

Hemolymph and digestive tract fluids were monitored for spiroplasmas by phase-contrast or dark-field microscopy at ×1000. Hemolymph samples were taken by puncturing intersegmental membranes at the base of the prothoracic legs with fine glass capillary tubes. Dissected digestive tracts were rinsed in three changes of sterile 0.9 percent saline solution and then transferred to Singh's mosquito tissue culture medium (SM-1) (*16*). Segments 2 to 3 mm in length from the anterior, middle, and posterior portions of the midguts were compressed between slides and cover slips and examined microscopically. Specimens were prepared for electron microscopy as described earlier (*17*). Transmission experiments were performed by feeding or injecting cultured organisms suspended in medium. Spiroplasmas that did not grow in SM-1 or in other media were transmitted to noninfected insects by injection or by feeding hemolymph or filtered (0.45-μm pore) gut suspensions from infected insects.

Antiserums (*18*) produced against spiroplasmas representing serogroups (*19*) I-1, I-2, I-3, I-4, III, IV, V, and VI (*20*) were used in serological deformation tests (*21*) to type cultivated spiroplasmas from the bumble bee (BI-1), wasp (MQ-1), and syrphid fly (EA-1) and to type the Colorado potato beetle spiroplasma (CPBS) collected from intestines of infected beetles.

Spiroplasmas seen in the hemolymph of five species of Hemiptera (Table 1) have not as yet been classified serologically because they failed to grow in culture media [M1A (*22*), SP-4 (*10*), or SM-1 (*11*)] that support growth of other spiroplasmas. Attempts to infect honey bees with hemipteran spiroplasmas also failed. It is possibly significant that four of the five hemipterans with spiroplasmas in their hemolymph were predators. The fifth hemipteran, *Lygus lineolaris* (tarnished plant bug) is a classical pest of a wide variety of crops and wild plants.

The CPBS was maintained in the laboratory by placing field-collected adults and larvae in screen cages 30 by 30 by 30 cm and supplying them with bouquets of potato leaves or tomato plants. Infected beetles could be identified by microscopic examination of the dark-brown regurgitated fluids of either larvae or adults or by examination of their semisolid fecal pellets. Pathogenicity of the CPBS was assessed by comparing survivals of 30 newly hatched larvae fed individually with suspensions of 10^8 organisms per milliliter of SM-1 medium with larvae fed medium only. After 24 hours, regurgitated gut fluid was examined microscopically for spiroplasmas. Larvae were then placed on young tomato plants in a greenhouse held at 30° to 35°C. Survival of test and control insects was compared after 2 weeks, at which time most larvae had pupated.

Adult Colorado potato beetles that had emerged from their overwintering sites in the ground were noticed on the leaves of young potato plants during the last week in May 1981. Twenty-five adult beetles were examined, and 76 percent were found to harbor spiroplasmas. These first estimates of infection incidence were probably low because spiroplasmas were initially recognized by their size, helicity, and characteristic

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motility. Later, large numbers of granular masses in the midguts also were found to be composed of spiroplasmas. These aggregates, which ranged from about 5 μm to more than 100 μm in diameter, usually showed no signs of motility while bathed in digestive fluid, but rapidly disaggregated into extremely active helical cells when flooded with SM-1. Because these masses were often found in beetles in which no motile spiroplasmas were observed, many infected beetles could have been recorded as not infected. The incidence of infection among the first-generation fourth instar larvae remained rather low throughout June (about 5 percent). However, when larvae of all instars were caged with field-collected adults, they usually became infected within 2 days. Beetles of all ages were also readily infected by feeding them either fecal pellets or gut contents containing spiroplasmas dispersed in SM-1. No spiroplasmas were found in hemolymph from 170 adults or 120 larvae. Forty adult and 40 fourth instar larvae were injected intrahemocoelically with 5×10^5 spiroplasmas but did not develop hemolymph infections. Therefore, the infection appears to be limited to the gut lumen. Spiroplasmas were not found in newly molted larvae or in pupae, perhaps because the digestive tract of the beetle host is normally evacuated before both molting and pupation. Newly molted larvae quickly became reinfected, however, either by devouring their shed skins or by ingesting contaminated leaves. In the pathogenicity test there were no significant differences in survival between test and control larvae.

When tested by deformation against antiserums having a titer of 1:5120 for the Maroc strain of *Spiroplasma citri*, the CPBS gave a positive reaction at 1:20, but failed to react with serums directed against other spiroplasma groups or subgroups. Such low orders of cross-reactivity are difficult to interpret. On the other hand, deformation tests showed that bumble bee isolates BI-1 and BI-2 are serologically indistinguishable from the honey bee spiroplasma (BC-3 isolate), and can definitely be assigned to serological subgroup I-2.

The spiroplasma from the vespid wasp, *Monobia quadridens* (MQ-1), was similar to that of the honey bee in that midgut infections were also accompanied by hemolymph infections. However, numbers of spiroplasma cells in the hemolymph of the wasp were always rather low (highest was 7×10^7 per milliliter) compared with achievable titers of 10^{10} in the honey bee. Also, the frequency of infections among the wasps was more than 90 percent ($N = 36$), which is much higher than in any honey bee population examined even if one considers only foraging bees. Serologically, MQ-1 was found to be distinct from existing spiroplasma groups or subgroups. Neither signs nor symptoms of disease were evident among the infected *M. quadridens*.

Table 1. Occurrence of spiroplasmas in diverse insect hosts.

Insect species	Specimens	
	Examined (No.)	Infected (No.)
Order Hymenoptera		
Family Apidae		
Bombus impatiens	381	3
Family Vespidae		
Monobia quadridens	38	33
Order Diptera		
Family Syrphidae		
Eristalis arbustorum	3	1
Family Asilidae		
Two	3	3
Order Hemiptera		
Family Reduviidae		
Pselliopus cinctus	3	3
Rocconoto annulicornis	1	1
Family Rhopalidae		
Arhyssus lateralis	3	2
Hormostes reflexulus	2	1
Family Miridae		
Lygus lineolaris	15	3
Order Coleoptera		
Family Chrysomelidae		
Leptinotarsa decemlineata	200+	About 85 percent

The spiroplasma (EA-1) from the syrphid, *Eristalis arbustorum*, is the second to be reported from the order Diptera. Unfortunately, spiroplasma cells were not noticed when the hemolymph was examined by light microscopy; they were probably overlooked because of the presence of a very active trypanosomatid flagellate. Fortuitously, although the medium that was chosen (*23*) did not support growth of the trypanosomatid, it did permit growth of the spiroplasma. Flagellates are found only rarely in insect hemolymph, and their presence in the insect examined suggests that the gut may have been ruptured; thus the natural habitat of the EA-1 spiroplasma could be the insect intestine, rather than the hemolymph. Deformation tests have shown that this spiroplasma reacts very weakly with spiroplasmas of group I, but is distinct from the existing subgroups of the group I complex.

Spiroplasmas were also seen in the midguts of two other species of flies, both of the predaceous family Asilidae. The examination procedure destroyed the specimens. No attempt was made to cultivate the spiroplasmas from either host species. It was not determined whether these predators had recently consumed an infected insect or whether their digestive tracts had actually been colonized by the spiroplasmas.

Until now, concepts of spiroplasma ecology have been based on complex relationships that regularly involve the hemolymph of leafhoppers and honey bees. For example, because the honey bee spiroplasma (BC-3 and related strains) was initially found in the hemolymph of its host (*11*) and experimental feeding produced hemolymph infections, it was assumed, perhaps erroneously, that the hemolymph was the normal habitat of spiroplasmas in insects and that the midgut merely served as a site of invasion. Further studies of the host-parasite relationship of the honey bee spiroplasma strain BC-3 have now been conducted, and confirm a greater complexity than that for the CPBS. Cells of the BC-3 organism appeared in the blood of newly emerged bees 7 to 14 days after oral administration of doses of 7×10^6 organisms. Death of the bees usually followed hemolymph invasion within about 3 days. Midgut infestations were found in most experimentally infected honey bees, and also in some naturally infected foraging bees. Thus, hemolymph infections were probably preceded by colonization of the midgut of foraging bees. However, in July, when the incidence of spiroplasmas in foraging bees was usually less than 5 percent, some infected bees were apparently free of gut infestations. Spiroplasmas may therefore persist longer in the blood than in the gut of at least some foragers, suggesting a possible role of hemolymph infection in natural maintenance.

Discovery of the CPBS, which clearly exists in a gut-plant surface cycle, clarifies and extends our understanding of spiroplasma-insect interactions. The life cycles of many spiroplasmas may be basically similar to that of the CPBS. In such infections, in contrast to that of the honey bee in which the hemolymph may normally be invaded, organisms in the hemolymph probably play no role or, perhaps, if they are pathogenic, even a negative role in the maintanence of the spiroplasma in nature. Hemolymph infections with these intestinal commensals may result only when the organisms wander from their normal habitat by penetrating the gut wall and enter the hemolymph of their hosts. While the mode of maintenance of the CPBS may be considered the simplest and perhaps

the most primitive among the spiroplasmas now known, those of the *Drosophila* sex-ratio spiroplasmas (*6*) might be considered the most highly evolved. These organisms, apparently lacking a gut phase, may be wholly dependent on transovarial transmission for their survival, although transmission by mouth has been reported in one species (*24*).

We cannot yet compare the ecology of spiroplasmas from insects with those of other arthropods such as ticks. Although two tick genera have been reported to harbor spiroplasmas (*20*), the maintenance cycles of the organisms have not been elucidated. Knowledge of the host-parasite relationships of tick spiroplasmas might provide evidence concerning the existence of alternative vertebrate hosts.

Thus it is clear that a diversity of spiroplasma-insect relationships exist. However, the contamination of plant surfaces by feces or regurgitated gut contents from insects infected with spiroplasmas is an important means of spreading infections in nature. This conclusion is supported by the observation that a spiroplasma found in the bettle, *Melolontha melolontha* in Europe (*25*) is serologically identical to spiroplasmas of group III (*26*). This group of strains includes strain 23-6 of Davis (*27*) and BNR1 of Clark (*12*), both from the tulip tree (*Liriodendron tulipifera*) and OBMG of Clark (*12*) from the southern magnolia (*Magnolia grandiflora*). The name *Spiroplasma floricola* has been proposed for these strains (*28*). Although many spiroplasmas may not be pathogenic to their usual insect hosts, their exceptional stability on various surfaces (*29*) and wide experimental host ranges suggest that some may have application for biological control.

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Differential Breakdown of Phylogenetically Diverse Ribosomal RNA's Inserted via Liposomes into Mammalian Cells

Abstract. *Liposomes were used to deliver ribosomal RNA's from three different organisms into cultivated mouse plasmacytoma cells. Ribosomal RNA from* Escherichia coli *was degraded intracellularly within 1 hour, whereas mouse and yeast ribosomal RNA's were degraded more slowly. This indicates that cells can discriminate between different ribosomal RNA's.*

Although the regulation of protein synthesis is controlled mainly at the level of messenger RNA (mRNA) synthesis, regulation may also occur at the posttranscriptional level, by modulation either of the initiation of translation (*1*) or of the stability of RNA (*2*). Any method that would allow direct delivery of mRNA into cells and permit a study of the differential processing of RNA molecules in an unmodified environment would facilitate the investigation of posttranscriptional regulatory mechanisms.

In recent years lipid vesicles (liposomes) have been used to deliver biologically active macromolecules into a variety of eukaryotic cells in vitro (*3*). The liposomes protect the sequestered molecules from external agents and can deliver macromolecules to a large number of cells. In particular, liposome-sequestered RNA is protected from the action of ribonuclease (*4*). Translation of globin mRNA (*5*), infectivity of polio RNA (*6*), and association of myosin mRNA with ribosomes (*7*) have been demonstrated in foreign cells into which the appropriate RNA was injected via liposomes. We have shown that a substantial amount of liposome-entrapped RNA can be delivered into cells and that most of the cell-associated RNA is internalized (*8*). We now describe experiments indicating that liposome-delivered ribosomal RNA's (rRNA's) of different phylogenetic origin are degraded at different rates. Because rRNA in vivo is usually bound to proteins, these experiments were not intended as a means of studying the degradation of rRNA in vivo, but rather were performed to assess whether this technology could be used to investigate the putative differential processing of RNA molecules.

Total RNA from *Escherichia coli* A 155 (U^-) was labeled with ^3H or ^{32}P (*4*). Mouse rRNA labeled with ^{32}P was prepared from P3XAg6593 cells (derived from the myeloma MOPC21) grown for 24 hours in Eagle's minimal essential medium containing one-tenth the normal amount of inorganic phosphate and supplemented with [^{32}P]phosphate (10 to 20 μCi/ml). Cells were lysed in a buffer containing 10 m*M* sodium acetate (*p*H 5.1), 100 m*M* NaCl, 1 m*M* EDTA, and 0.5 percent sodium dodecyl sulfate and extracted with a phenol-chloroform mixture (1:1). The total nucleic acid was precipitated with ethanol and repeatedly suspended in 3*M* sodium acetate (*p*H 6.0) to solubilize 4*S* to 5*S* RNA and

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