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Vector–Spirochete Relationships in Louse-Borne and Tick-Borne Borrelioses with Emphasis on Lyme Disease

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Introduction

The discovery in 1981 of the spirochete, now known as *Borrelia burgdorferi*, as the etiological agent of Lyme disease in the United States and of associated clinical manifestations in Europe (11, 12) has rekindled interest in arthropod-borne spirochetoses. Since then, hundreds of publications have appeared that deal not only with the complex clinical aspects of Lyme disease but also with the natural history of this agent, and particularly its relationship to its arthropod vectors—ticks of the genus *Ixodes*. In the United States, where Lyme disease is now considered the most prevalent tick-borne disease (16), *B. burgdorferi* is associated with at least three *Ixodes* species, namely *Ixodes dammini* in the northeastern and midwestern regions of the country, *Ixodes pacificus* in the West, and possibly *Ixodes scapularis* in the Southeast. In Europe (55), the sheep or castor bean tick, *Ixodes ricinus*, is the vector, whereas in Asia *Ixodes persulcatus* is said (17) to be involved in the maintenance and distribution of the Lyme disease spirochete.

Borrelia burgdorferi may have eluded investigators since the beginning of the century because of its unique parasite–vector relationship, which, as we shall see, differs from that of most other arthropod-borne spirochetes, and because ticks of the genus *Ixodes* had never been suspected to serve as vectors of spirochetes.

Before the 1981 discovery of *B. burgdorferi* in *Ixodes* ticks (11), most arthropod-borne spirochetes, as summarized in Table 5.1, were known to

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TABLE 5.1. Characteristics and distribution of arthropod-borne borreliae.[a]

Borrelia sp.	Arthropod vector	Animal reservoir	Distribution	Disease
B. recurrentis (syn. *B. obermeyeri, B. novyi*)	*P. humanus humanus*	Humans	Worldwide	Louse-borne, epidemic relapsing fever
B. duttonii	*O. moubata*	Humans	Central, Eastern, and Southern Africa	East African tick-borne, endemic relapsing fever
B. hispanica	*O. erraticus* (large variety)	Rodents	Spain, Portugal, Morocco. Algeria, Tunisia	Hispano-African, tick-borne relapsing fever
B. crocidurae, B. merionesi B. microti, B. dipodilli	*O. erraticus* (small variety)	Rodents	Morocco, Libya, Egypt, Iran, Turkey, Senegal, Kenya	North African, tick-borne relapsing fever
B. persica	*O. tholozani* (syn, *O. papillipes, O. crossi*?)	Rodents	From West China and Kashmir to Iraq and Egypt, USSR, India	Asian-African, tick-borne relapsing fever
B. caucasica	*O. verrucosus*	Rodents	Caucasus to Iraq	Caucasian, tick-borne relapsing fever
B. latyschewii	*O. tartakowskyi*	Rodents	Iran, Central Asia	Caucasian, tick-borne relapsing fever
B. hermsii	*O. hermsi*	Rodents, chipmunks, tree squirrels	Western United States	American, tick-borne relapsing fever
B. turicatae	*O. turicata*	Rodents	Southwestern United States	American, tick-borne relapsing fever

B. parkeri	*O. parkeri*	Rodents	Western United States	American, tick-borne relapsing fever
B. mazzottii	*O. talaje* (*O. dugesi*?)	Rodents	Southern United States, Mexico, Central and South America	American, tick-borne relapsing fever
B. venezuelensis	*O. rudis* (syn. *O. venezuelensis*)	Rodents	Central and South America	American, tick-borne relapsing fever
B. burgdorferi	*I. dammini*	Rodents	Eastern United States	Lyme disease and related disorders
	I. pacificus	Rodents	Western United States	Lyme disease and related disorders
	I. ricinus	Rodents	Europe	Lyme disease and related disorders
	I. persulcatus	Rodents	Asia, China, Japan	Lyme disease and related disorders
	Possibly other hematophagous arthropods	Possibly other reservoirs (deer, birds)	Worldwide(?)	Lyme disease and related disorders
B. coriaceae	*O. coriaceus*	Rodents (deer?)	Western United States	Epizootic bovine abortion (?)
B. theileri	*Rhipicephalus* spp. *Boophilus* spp.	Cattle, horses, sheep (?)	Worldwide	Bovine borreliosis
B. anserina	*Argas* spp. (mites?)	Fowl	Worldwide	Avian borreliosis

[a]Spirochete-tick associations of unknown or little health significance not included.

be associated with soft-shelled ticks of the genus *Ornithodoros* or *Argas*. Exceptions were *Borrelia recurrentis,* the louse-borne, relapsing fever spirochete, and *Borrelia theileri,* a spirochete maintained and distributed among cattle, horses, and sheep by ticks of the genus *Rhipicephalus* and *Boophilus*.

After the human body louse *Pediculus humanus* (53) and the African soft-shelled tick *Ornithodoros moubata* (20) were found to be the vectors of the relapsing fever spirochetes (known today as *Borrelia recurrentis* and *Borrelia duttonii,* respectively) the development of these microorganisms in their vectors and the modes of their transmission to humans, was intensively studied. The purpose of this chapter is to review the most salient findings of these studies and to compare them with observations made so far on *B. burgdorferi,* in its tick vectors.

The Behavior of Louse-Borne and Tick-Borne Spirochetes in Their Vectors

Shortly after Sergent and Foley (53) confirmed that the human body louse *P. humanus humanus* was the vector of the European relapsing fever spirochete, in 1910, Nicolle et al. (45) studied the behavior of a North American strain of *B. recurrentis* in lice and noted that the spirochetes had disappeared from the midgut 24 hours after they had been ingested; they were not detectable again until days six through eight, when they suddenly began to reappear in the hemolymph. A similar "negative phase" for *B. duttonii* in *O. moubata* had been observed previously by several investigators, including Dutton and Todd (21), Leishman (38), Fantham (22), and Hindle (33) and later also by Hatt (30) and Nicolle et al. (44).

These authors found that ingested spirochetes invade the gut epithelium, where they lose their motility and, after three to four days, develop into spherules or gemmae that contain varying numbers of granules or chromatin bodies. According to Dutton and Todd (21), these spherules are formed by a protuberance (aneurysm) of the periplasmic membrane; they may form at any point along the spirochete. Some time after the spherules form, they are said to burst and to release their contents. The tenth day after an infectious feeding, morphologically typical spirochetes were no longer found (21); Dutton and Todd found large numbers of granules from which new spirochetes eventually developed, provided the ticks were maintained at temperatures above 25 °C.

Hindle (33) reported similar findings. In infected ticks held at 21 °C, the spirochetes disappeared from the midgut by the tenth day after infectious feeding. They could no longer be detected either in the gut or in the tissues of various organs. Triturates of such ticks injected into mice, however, regularly proved infectious, and an increase in temperature to 35 °C led to the reappearance of morphologically typical spirochetes.

This "granulation theory" received a significant boost in 1950, when Hampp (29), of the National Institute of Dental Research, showed by stained smears and darkfield and electron microscopy that oral treponemes and *Borrelia vincenti* in cultures produced blebs and granules, which he considered to be possible germinative units. His hypothesis was supported by the observation that 31-month-old cultures containing only granules invariably produced typical spirochetes upon transfer to fresh medium.

Similar observations were also reported by DeLamater et al. (18). Their data supported the occurrence of a complex life cycle in the pathogenic and nonpathogenic strains of *Treponema pallidum*. This spirochete was said to multiply (a) by transverse or binary fission and (b) by the production of gemmae in which either a single granule or masses of granules appeared to be the primordia of daughter spirochetes.

Many investigators, including Wittrock (59), Kleine and Eckard (35), Kleine and Krause (34), Feng and Chung (23), and Burgdorfer (9), who conducted studies on the developmental dynamics of various species of borreliae in lice or ticks, however, found no evidence of a "negative" phase. Although most of these authors verified the existence of blebs or gemmae on spirochetes, they considered them to be products of degeneration—a conclusion also reached by Pillot et al. (48), who published an extensive electron microscopic study of these degenerating forms on cultured *T. pallidum, B. duttonii, B. hispanica,* and *Leptospira icterohaemorrhagiae*.

In 1947, Garnham et al. (26) suggested that louse-borne spirochetes could be divided into those that have a negative phase (Europe, North Africa, and Kenya) and those that do not have a negative phase (Abyssinia and China). In 1962, however, Heisch—who had worked with Garnham in 1947—and Harvey (31) called the negative phase an artifact. Thus, the question of a complex developmental cycle for borreliae appeared settled, and it was generally accepted that *B. recurrentis* spirochetes in the body louse *P. humanus humanus,* after ingestion of a patient's blood, arrive in the midgut where most are destined to die. Those that survive pass through the gut wall into the hemolymph where they multiply by binary fission. They then invade the neural ganglia and the muscles of the head and thorax. They were never observed in the salivary gland tissues or in other organs. Thus, the transmission of *B. recurrentis* to humans does not occur by bite, that is, via saliva, but rather by contamination of the bite wound with infectious hemolymph of lice crushed or wounded by a person's scratching.

Similarly, *Ornithodoros moubata* and other relapsing fever ticks, during their mostly short feeding (10 to 30 minutes), ingest spirochetes via the pharynx and esophagus into the midgut where they can be found in gradually decreasing numbers for about 14 days. Within hours after a blood meal, spirochetes accumulate in the intercellular spaces of the

tick's gut epithelium. As early as 24 hours after ingestion, they penetrate the basement membrane to enter the body cavity where they undergo intensive multiplication by binary fission. From here, the spirochetes invade the various tissues, particularly those of the central ganglion, the coxal organs, and the genital system. They also thrive in the connective tissue surrounding the Malpighian tubules. Only in nymphal ticks do salivary gland tissues also become heavily infected. Once an infected *O. moubata* reaches the adult stage, the salivary glands are spirochete-free, or only mildly infected. Thus, the mechanisms for transmission of *B. duttonii* by *O. moubata* vary, and depend on the developmental stage of the tick. Nymphs transmit by spirochetes (a) bite, that is, via spirochete-containing saliva, and by (b) spirochete-containing coxal fluid, excreted shortly before feeding ends, which washes the spirochetes directly into the bite wound. Adult ticks, however, transmit spirochetes primarily by infected coxal fluid, and only rarely by infectious saliva (9).

The invasion of spirochetes into germinal cells, that is, oogonia and early oocytes, invariably leads to transovarial infection, with filial infection rates as high as 90%. It is said (1) that spirochetes remain relatively passive during the early phases of oogenesis but leave the ooplasm to invade the developing embryonic nervous tissue system. Only from this tissue do spirochetes invade other tissues, such as the salivary glands, during the tick's postembryonic stage.

The attraction of spirochetes to nervous system tissue of their arthropod vectors has been observed for *B. recurrentis* in the body louse and also for many Ornithodoros–spirochete associations. In fact, microscopic examination of central ganglion tissues is by far the most dependable way to determine the infection status of field-collected, relapsing fever ticks.

The dynamic development of spirochetes, which is similar to that outlined above for *B. recurrentis* in *P. humanus humanus* and for *B. duttonii* in *O. moubata,* has been also recorded for other tick–spirochete associations, including *Borrelia crocidurae* in *Ornithodoros erraticus* (25) and *Borrelia anserina* in *Argas persica,* and three other *Argas* spp. (19, 60). For most relapsing fever tick–spirochete associations, numerous references describe the vector's efficiency in transmitting spirochetes and in passing borreliae transovarially, that is, via eggs to the progeny (10). But vertical transmission does not occur in every tick–spirochete association. It has been reported for the genus *Ornithodoros,* in *O. moubata, O. erraticus* (both varieties), *O. tholozani, O. tartakovskyi, O. verrucosus, O. turicata,* and *O. hermsi,* but not in *O. parkeri, O. talaje,* and *O. rudis.* The percentage of infected female ticks passing spirochetes via eggs (transovarial transmission rate) varies greatly, as does the percentage of filial ticks (filial infection rate) that become infected. The efficiency of these phenomena appears to depend on the degree of spirochetal infections in ovarial tissue and germinal cells.

Although transovarial transmission is an effective way to infect ticks, it may render strains of borreliae nonpathogenic if they are maintained continuously without occasional passage in a susceptible host (28). This was considered responsible for a dramatic drop in the incidence of relapsing fever in Tanzania, where more than 20% of *O. moubata* carried spirochetes, that were once highly pathogenic for laboratory animals but were now no longer capable of infecting them. The same phenomenon was also reported for certain strains of *Borrelia sogdiana* after eight successive generations in *O. tholozani* (5).

Although transovarial transmission of tick-borne relapsing fever spirochetes is common, venereal transmission from male to female ticks during copulation appears to be rare. According to Wagner-Jevseenko (57), *B. duttonii* could occasionally be demonstrated in the fluid of spermatophores, but in only 2 of 96 females who mated with infected males did the sexually transferred spirochetes produce infections. In contrast, Gaber et al. (24), studying venereal transfer of *B. crocidurae* in Egyptian *O. erraticus,* concluded that polygamous males contribute significantly to the spread of the spirochete in this tick vector. After the first and second gonotropic cycles, spirochetes were observed in 23 and 37% of female ticks, respectively. Although the mechanism(s) of transmission was not clear, the authors speculated that infected fluids from the males' genital accessory glands or infected saliva secreted during copulation or both played a role.

The Behavior of *Borrelia theileri* in Its Tick Vectors

Borrelia theileri, the etiological agent of bovine borreliosis, is prevalent in Africa, India, Indonesia, Australia, and South America, where it is associated with ixodid ticks, including *Boophilus microplus, Boophilus annulatus, Boophilus decoloratus,* and *Rhipicephalus evertsi.* An even wider geographical distribution has been suggested by the demonstration of *B. theileri* or similar spirochetes in the blood of cattle in North America and Europe (56).

Little information is available on the relationship of this spirochete to its tick vectors, in which it produces a systemic infection, with the ovary and central ganglion most consistently infected. Transovarial transmission with filial infection rates as high as 80% does occur, but the larval ticks seem to be incapable of transmitting the spirochetes. (The reader is reminded that ticks of the genus *Boophilus* are one-host ticks, i.e., all stages and molts occur on the same host animal.) According to Smith et al. (54), who studied the development of *B. theileri* in the progeny of field-collected *B. microplus* from Mexico, spirochetes in ovarially infected larvae, 22 hours after placement on a calf, were few in number but increased in feeding nymphs and adults, especially after repletion. The authors also noted a massive growth of organisms in hemocytes and the

release of spirochetes from these cells into the hemolymph, where intensive multiplication resulted in masses of spirochetes. These heavy spirochetal infections of hemolymph, ovary, and other tissue did not alter the ticks' feeding or reproductive habits.

The Behavior of the Lyme Disease Spirochete *Borrelia burgdorferi* in Its Tick Vectors

Unlike the spirochetes of relapsing fever and bovine borreliosis that leave the midgut of their vectors shortly after ingestion, *B. burgdorferi* spirochetes, in most of their tick vectors, remain primarily in the midgut, where they aggregate near the microvillar brushborder and in the intercellular spaces of the gut epithelium. From there, they may penetrate the gut wall during or after engorgement to initiate mild systemic infections, particularly in tissues of hypodermis, ovary, and central ganglion and within muscle tissue associated with Malpighian tubules and tracheae (14).

Although our 1981 collection of flat, adult *I. dammini* from Shelter Island, New York yielded ticks with midgut infections only—77 (61%) of 126 ticks—subsequent examination of 151 additional unfed adult ticks from the same region revealed 102 with midgut infections. Of these, four (3.9%) were systemically infected (14). Similar results were obtained with the European tick vector *I. ricinus*. Of 112 infected flat adults collected from vegetation in western Switzerland, 106 (91%) had midgut infections only. The remaining six had spirochetes throughout their tissues (12). A somewhat larger percentage of ticks with systemic infections was recorded for the western black-legged tick *I. pacificus,* from northern California and southwestern Oregon. Of 1678 ticks examined, 25 (1.5%) had spirochetes in their midgut. Of these, eight (32%) were also systemically infected (15).

The percentage of ticks with spirochetes throughout their tissues increased when examinations were done after the ticks had been allowed to feed. Thus, of 46 midgut-infected *I. dammini* females evaluated six to eight weeks after repletion, 9 (19.5%) had spirochetes throughout their tissues. Similarly, of 25 engorged females that had failed to oviposit for more than three months after feeding, 23 (92.9%) had midgut infections, and 18 (78.2%) of these 23 had systemic infections (14).

Thus, it appears that "gut penetration" by *B. burgdorferi* is closely associated with histological changes in the gut epithelium during or after feeding or both. Preliminary transmission and scanning electron microscopy studies suggest that *B. burgdorferi* spirochetes aggregate within clefts or pitlike structures between epithelial cells and through them become closely associated with the basement membrane (Figures 5.1 and 5.2). Actual penetration of this membrane has not yet been observed.

According to Ribeiro et al. (50), penetration of the gut epithelium and

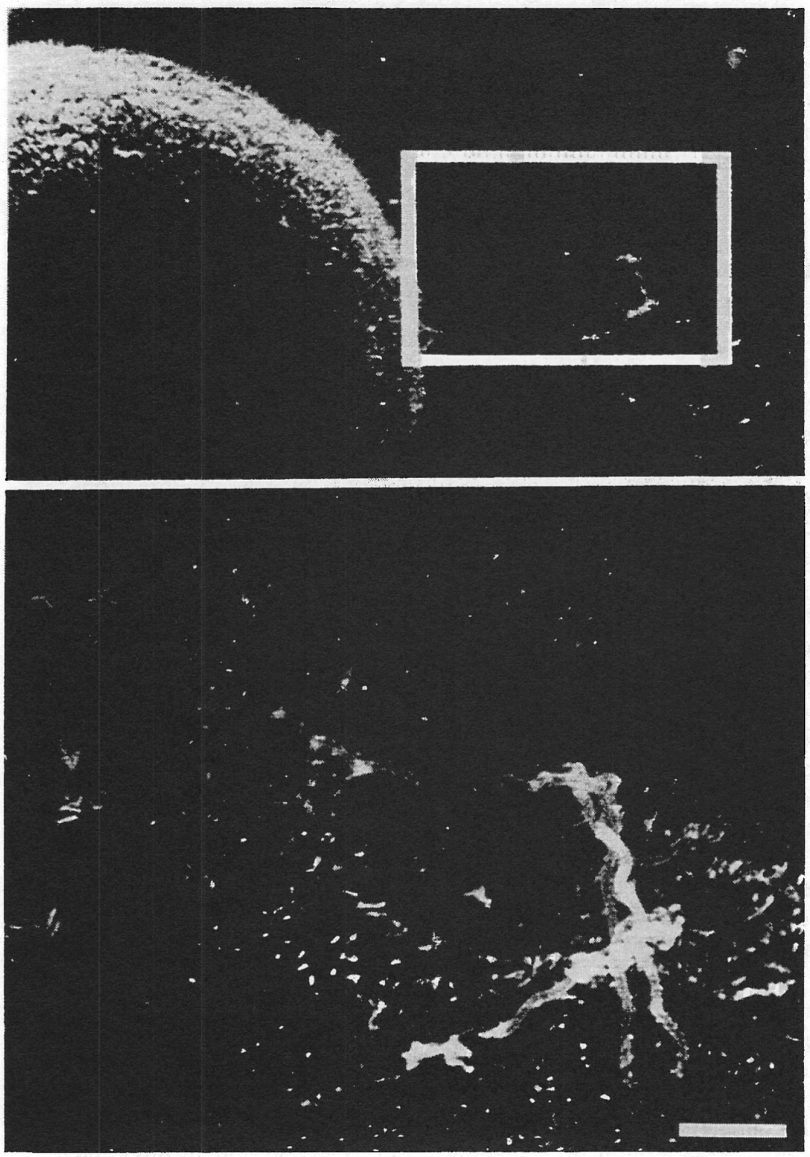

FIGURE 5.1. Association of *Borrelia burgdorferi* with a pitlike structure in the midgut epithelium of an infected *Ixodes dammini* (scanning electron micrograph; bar, 1.0 μ). (Reprinted with permission from *Reviews of Infectious Diseases*.)

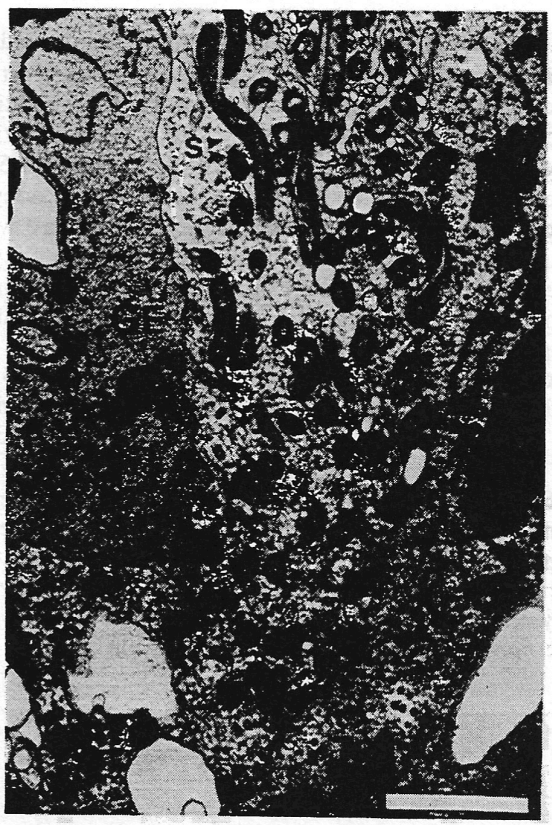

FIGURE 5.2. *Borrelia burgdorferi*-filled pit in the midgut eipthelium of an infected *Ixodes dammini*. s (arrows), typical serpentine profile of spirochete; DE, gut epithelial cell (transmission electron micrograph; bar, 1 μ). (Reprinted with permission from *Reviews of Infectious Diseases*.)

spirochete dissemination takes place during the first few days the tick is attached. By the fifth day, 20 of 35 midgut-infected *I. dammini* had spirochetes in their hemolymph. Based on their findings of sequential histological studies, Benach et al. (6) drew a similar conclusion and suggested that *B. burgdorferi* multiplies in midgut tissues during early feeding (days 1–3) and penetrates the gut wall during midfeeding (days 5–7), to enter the hemocoel. From there, the spirochetes disseminate via hemolymph to other tissues, particularly the central ganglion.

In systemically infected *I. dammini,* the Lyme disease spirochete, although present in the midgut, usually only mildly infects other tissues. It is rarely found in the hemolymph, which, unlike that of the relapsing fever and bovine borreliosis vectors, does not appear to be an optimal medium

for its development. Massive spirochetal infections have been found in ovarian tissues of engorged *I. dammini* females that failed to oviposit or that laid small batches of eggs only (14). Electron microscopic evaluation of such tissues showed intensive spirochetal invasion of supporting tissue, as well as of developing oocytes. As illustrated in Figure 5.3, numerous spirochetes may be present between the vitelline and oocyte membranes, where they denude the oogonial surface and interfere with the transport of chitin, which is necessary for the formation of the oocyte shell; eggs so infected fail to mature and prevent ticks from ovipositing.

Nevertheless, demonstration of *B. burgdorferi* in field-collected, unfed larval *I. dammini* suggests that mild spirochetal infections of oocytes do permit the eggs to develop (7, 46). Recently, infection rates of 3.3 to 15.0% were reported in the progeny of five female ticks removed from a deer, and of 25 and 27% in the progeny of two female ticks from a dog (42).

Experimentally infected *I. dammini* were also found to pass spirochetes via eggs, but decreasing infection rates in the larvae and the absence of spirochetes in nymphs suggested a gradual die-off of spirochetes; the number of ovarially transferred spriochetes may have been too low to initiate permanent systemic infections (42).

The passage of *B. burgdorferi* via eggs had been established also for

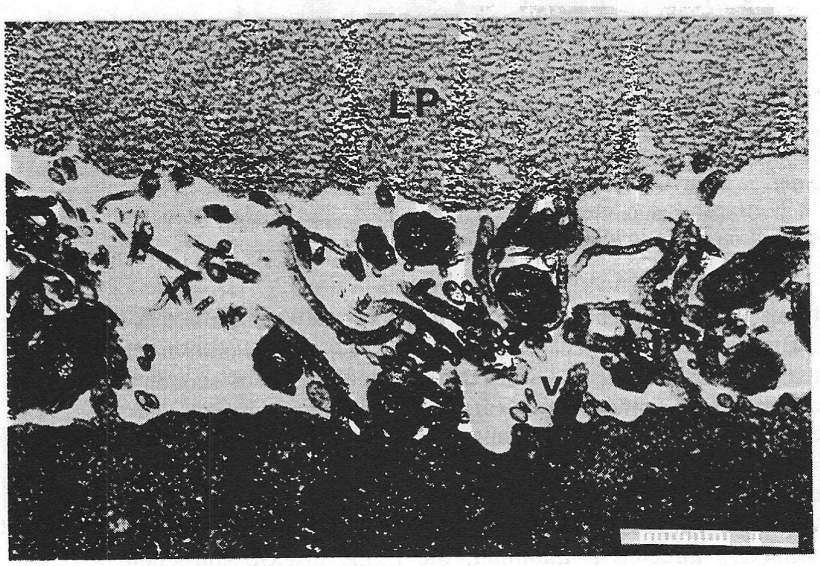

FIGURE 5.3. Cross section through a *Borrelia burgdorferi*-infected oocyte of an *Ixodes dammini* female with numerous spirochetes between the lamina propria (LP) and a oogonial cell (Oo). Note the destruction of the villar processes (v) (transmission electron micrograph; bar, 2.5 μ).

two naturally infected *I. ricinus* females from Switzerland. One female produced 100% infected eggs, the other only 60%; the F_1 larvae and nymphs were not examined (12).

So far, transovarial and subsequent transstadial passage of *B. burgdorferi* has been recorded only in *I. pacificus* (36). One of three naturally infected females produced 100% infected larvae that maintained the spirochetes transstadially; and four or five F_1 females passed them via eggs to as many as 97% of the F_2 progeny.

Of particular interest was the greatly reduced staining reaction of the ovarially passed spirochetes when they were treated with anti-*B. burgdorferi* FITC-labeled polyclonal antibody. Also, none of the spirochetes in the F_1 generation, reacted with a monoclonal antibody (H5332), which supposedly is specific for *B. burgdorferi*. Spirochetes in the F_2 generation, however, reacted.

Reduced immunofluorescence has been noted also for spriochetes in systemically infected *I. dammini* (W. Burgdorfer, unpublished information). Whereas the spirochetes in midgut tissues consistently showed strong fluorescence, those in tissues of the hemocoel often showed weaker fluorescence. This observation has been interpreted as indicating a loss of antigenic properties due to unfavorable physiological conditions for spirochetes outside the midgut. Indeed, perhaps the ability of *B. burgdorferi* to develop in tissues other than the midgut depends on its pathogenicity and interaction with the various tissues and varies from strain to strain and from one species of tick to another.

The mode(s) of transmission of *B. burgdorferi* by its tick vectors has been a controversial issue because fewer than 5% of naturally or experimentally infected. *I. dammini* are systemically infected. Thus, some investigators postulate transmission as occurring via saliva only, whereas others consider regurgitation to be an additional means of transmission. Supporters of the saliva hypothesis point out, as reviewed above, that the Lyme disease spirochete undergoes intensive multiplication during early feeding, then penetrates the gut wall to invade, via the hemolymph, various tissues, including the salivary glands. Ribeiro et al. (50) demonstrated spirochetes in the saliva as early as three days after the tick becomes attached and thereafter in increasing number. Of 20 hemolymph-positive, infected adult *I. dammini*, 11 had spirochetes in their saliva four days after attachment. Similarly, of seven infected nymphal ticks of the same species, three secreted spirochetes in their saliva as early as three days after attachment. In all these instances, spirochetes were found in saliva before rapid engorgement, which suggested that transmission occurs by the salivary gland route during the late-feeding period.

Despite extensive examination, Benach et al. (6) failed to find *B. burgdorferi* in the lumen of salivary glands or in associated tissues. Nevertheless, the presence of spirochetes in tick-feeding cavities in rabbit

skin suggested that transmission occurs either by salivation or by regurgitation of midgut contents. In a follow-up study by the same laboratory, Wheeler at al. (58), using monoclonal antibody (11G1), reported detecting spirochetal components in immunoblots of salivary glands of engorging ticks and concluded that *B. burgdorferi* is transmitted by *I. dammini* via saliva during the later stages of feeding.

The proponents of transmission through regurgitation point to recent publications by Hesse (32), who used a radioactive tracer (isotope ^{32}P) to demonstrate regurgitation to be a reflux of midgut contents in the argasid tick *Ornithodoros moubata,* and by Brown (8) who provided evidence that female *Amblyomma americanum* regurgitate gut material during feeding. In addition, the following observations and considerations favor this mode of transmission. In our laboratory, several successful transmission experiments were carried out with naturally or experimentally infected *I. dammini* in which no generalized distribution of spirochetes was observed. Also, if dissemination of *B. burgdorferi* occurs regularly during engorgement, a relatively large proportion of midgut-infected nymphs should develop systemic infections detectable in the adult stage. In Long Island, where Lyme disease is highly endemic, less than 5% of infected questing adult *I. dammini* had a generalized infection. Studies are now in progress to elucidate further how *B. burgdorferi* is transmitted, with an emphasis on saliva or gut material as sources of spirochetes.

The Relationship of *Borrelia burgdorferi* to Nonspecific Tick Vectors and Other Hematophagous Arthropods

Although *I. dammini,* and *I. pacificus* in the United States; *I. ricinus* in Europe; and *I. persulcatus* in European and Asian USSR, China, and Japan are recognized as the principal vectors of *B. burgdorferi,* identical spirochetes have been detected also in the lone star tick *Amblyomma americanum* (51, 52), the dog ticks *Dermacentor variabilis* (2, 3) and *Rhipicephalus sanguineus* (49), the rabbit ticks *Ixodes dentatus* (4) and *Haemaphysalis leporispalustris* (37), the rabbit dermacentor *D. parumapertus* (49), the woodrat tick *Ixodes neotomae* (37), the winter tick *D. albipictus* (3), and the black-legged tick *Ixodes scapularis* (40). So far, the only species claimed as an additional vector to humans is *A. americanum* in the Northeast (New Jersey), where all active stages of this tick are found to harbor spirochetes (51, 52).

Detection of *B. burgdorferi* in *D. variabilis* and *A. americanum* is of interest because attempts to infect these ticks experimentally have failed in at least two laboratories. At the Rocky Mountain Laboratories, we found that larval ticks of both species readily ingested spirochetes through feeding on infected rabbits. Organisms were detectable in up to 80% of freshly molted nymphs but not in nymphs that had fasted for three to six months (W. Burgdorfer, unpublished information). Similarly,

Piesman and Sinsky (47) found that the spirochetes did not survive molting processes to nymphs. These findings suggest that either the minimum dosage requirement, that is, the number of spirochetes necessary to cause persistent infections, was not met, or that these ticks are refractory to certain strains of *B. burgdorferi*. This may also be true for other species of ticks that sometimes harbor spirochetes temporarily after engorging on a spirochetemic host.

Detection of *B. burgdorferi* in *H. leporispalustris, I. neotomae,* and *D. parumapertus* and evidence of past or current spirochetal infections in black-tailed jack rabbits (*Lepus californicus californicus*) in northern California have long suggested that lagomorphs and their ticks are possibly involved in the ecology of the Lyme disease spirochete (37). More recently, spirochetes identified as *B. burgdorferi* were also isolated from 71 of 168 *I. dentatus* taken off naturally infected cottontail rabbits (*Sylvilagus floridanus*) in New York (4).

Very little information is available on the behavior of *B. burgdorferi* in rabbit ticks. All four infected ticks (2 of 174 *H. leporispalustris* and 2 of 10 *I. neotomae*) from black-tailed jack rabbits had midgut infections and at least three of them had systemic infections involving most tissues. One of the two *H. leporispalustris* oviposited eggs, of which 67% contained spirochetes (37). As yet, there is no information concerning the development of the Lyme disease spirochete in *I. dentatus*.

A potential vector in the southern and southeastern United States is the black-legged *I. scapularis,* another member of the *I. ricinus* complex. Infected experimentally, this tick has been shown (13, 47) to maintain and distribute *B. burgdorferi* as efficiently as does *I. dammini*. So far, only two specimens, both engorged females, have been found to be naturally infected. Spriochetes were also detected in two larvae that emerged from eggs deposited by each of these females (40).

The claim by some persons that they contracted Lyme spirochetosis as a result of insect rather than tick bites prompted several investigations of the role of mosquitoes, deerflies, and horseflies as vectors of *B. burgdorferi*. Surveys conducted in southcentral Connecticut from 1984 through 1987 led to the detection of spirochetes in four species of deerflies, in seven species of horseflies, and in four species of mosquitoes (39, 41). The prevalence of the infection varied, although it reached 21% for *Chrysops callidus* and about 7 to 8% for each of the four species of mosquitoes. The number of spirochetes detected in smears of foregut tissues varied from one to five to more than twenty-five. These findings led to experimental studies in which blood-sucking *Aedes canadensis* and *Aedes stimulans* were allowed to feed on Syrian hamsters (39). Although 9 of 71 and 2 of 30 mosquitoes, respectively, had *B. burgdorferi* in their head tissues, none of the hamsters became infected with spirochetes.

Three species of laboratory-reared mosquitoes (*Aedes aegypti, Aedes*

atropalpus, Aedes triseriatus) and the horsefly (*Tabanus nigrovittatus*) were also infected experimentally by allowing them to feed (through a lambskin membrane) upon beef blood mixed with BSK medium containing *B. burgdorferi* (43). Of 562 mosquitoes, 134 (23.8%) ingested spirochetes that survived in the digestive system for up to six days and could be demonstrated in preparations of head or midgut tissues for fourteen days. Similarly, of 57 *T. nigrovittatus*, 28 had living spirochetes in head and anterior digestive tract tissue. Two females harbored spirochetes for two to three days.

Even though these observations indicate that these insects may not be suitable hosts for *B. burgdorferi*, mechanical transmission as a result of their intermittent feeding habits cannot be ruled out and should be evaluated further.

Borrelia burgdorferi: Subject of a Complex Development Cycle?

The use of fluorescence, scanning, and transmission electron microscopy in studies of *B. burgdorferi* development in its tick vectors has consistently revealed this organism's ability to form vesicles along its length or at its terminal (Figure 5.4). These vesicles are similar to those described for relapsing fever borreliae (see above) and were at first considered

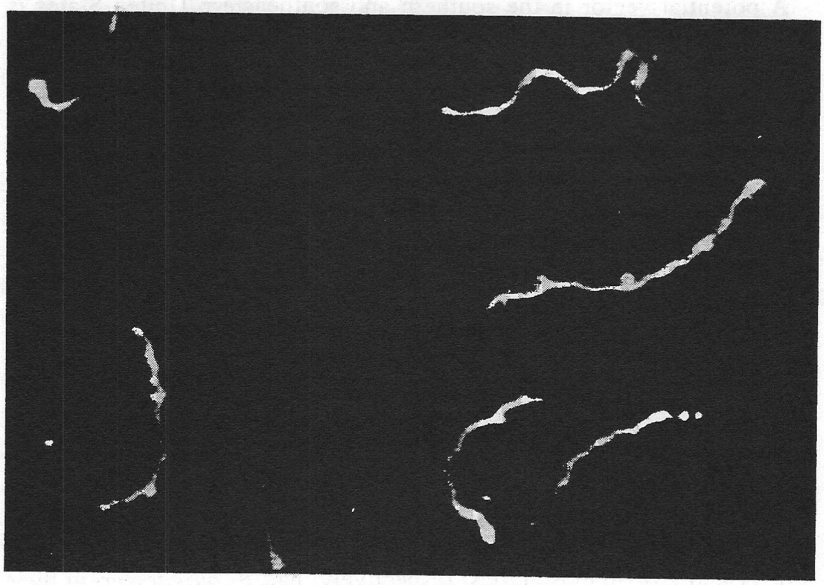

FIGURE 5.4. Fluorescent, antibody-stained *Borrelia burgdorferi* in a midgut smear of an infected *Ixodes dammini*. Note the gemmae (1330 ×).

germinative units, although later they were thought to be degenerative products.

In our experience, two types of vesicles can be identified: (a) "blebs," up to 200 nm in size, which develop as an eversion of the spirochete's outer membrane (Figure 5.5) and (b) "gemmae," up to 2.0 μ in size, with eversions not only of the outer membrane but also of the cytoplasmic cylinder (Figure 5.6). Some blebs do contain material of low electron density. Gemmae, however, usually contain one or more electron-dense chromatin bodies or granules.

Outer membrane blebbing appears to be an active process in *in vitro* and *in vivo* spirochetes. It results in a variety of structures that may be tubular (spaghetti-like) or string- or pearl-like or spherical (Figure 5.7). Gemmae are said to arise as the result of aging and/or of adverse changes

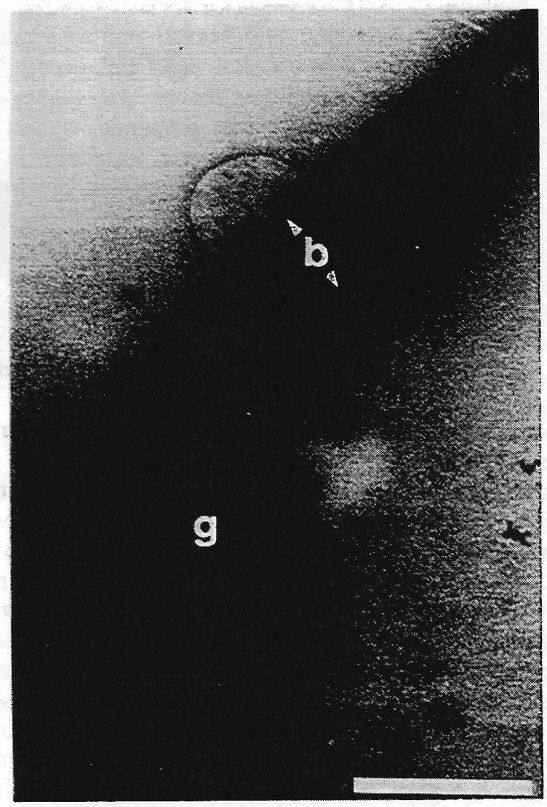

FIGURE 5.5. Negatively stained portion of *Borrelia burgdorferi* with eversion of the outer membrane (b) and the beginning of gemma (g) formation (bar, 0.5 μ).

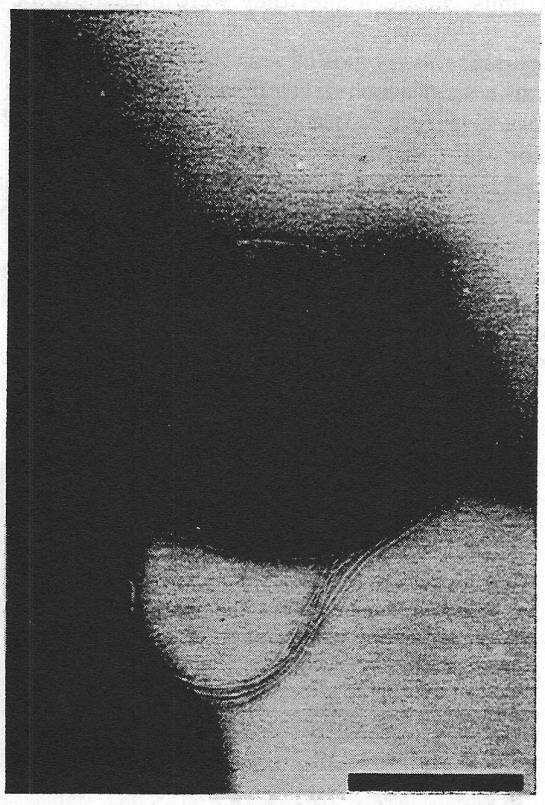

FIGURE 5.6. Negatively stained gemma (g) associated with a spirochete (bar, 1 μ).

in the spirochete's environment, such as changes in pH or depletion of metabolites (48).

In our laboratory, recent molecular investigations into the nature of these vesicles have shown that intact DNA is packed within some of them as well as within the granules of gemmae (27). Purified bleb preparations were found to contain genetic material in the form of linear and circular plasmids, which suggests that these vesicles may be involved in the exchange of genetic information. As yet, we have no evidence that the granules or chromatin bodies within gemmae give rise to a new generation of spirochetes, as has been postulated by some investigators (see above). However, we have been able to demonstrate encystment of spirochetes within elements of the outer membrane (Figure 5.8). As spirochetes begin to form gemmae, a part or, at times, the entire spirochete may coil and fold itself within its own outer membrane. A cross-sectional profile of such an entity would show a spherical unit containing several protoplasmic cylinders, as seen in Figure 5.9.

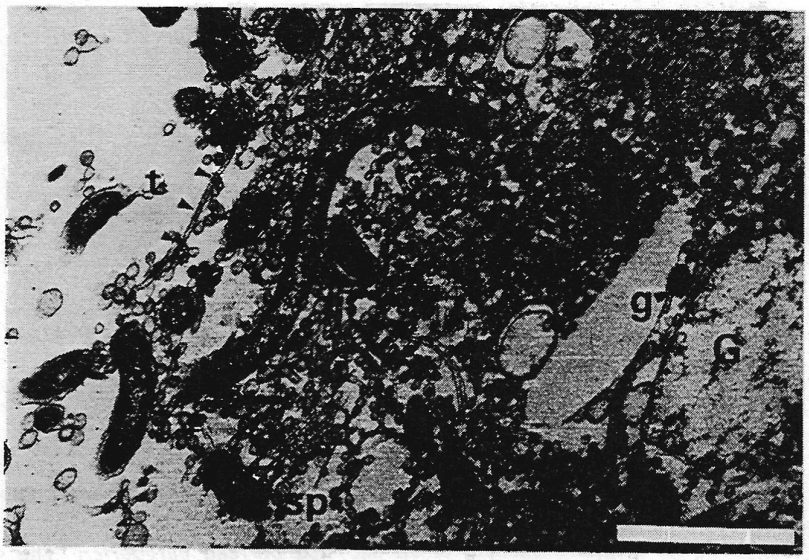

FIGURE 5.7. Extensive growth of *Borrelia burgdorferi* in the ovarial lumen of an infected *Ixodes dammini* female. Note the morphological variations of the spirochetal blebs. s, serpentine configuration of spirochete, t (arrows), tubular blebs; sp, string-of-pearl type blebs; G, empty gemma; g (arrow), granule (transmission electron micrograph; bar, 1.0 μ).

Immunochemical and molecular studies are in progress to shed additional light on the mechanism(s) of spirochetal development, which appears to be far more complex than was generally thought.

Conclusion

After spirochetes were discovered as the causative agents of louse-borne and tick-borne relapsing fevers, intensive studies on the development of these microorganisms in their vectors have shown that the ingested spirochetes leave the digestive tract to enter the hemocoel, where they undergo massive multiplication and then may invade the tissues of various organs. In the body louse, this development involves only the hemolymph and the nervous system's ganglia, whereas in the various tick vectors, it involves, with few exceptions, all organs.

With the discovery of a borrelia in ticks of the genus *Ixodes* as the etiological agent of Lyme borreliosis, a hitherto unknown tick–spirochete relationship was identified. The newly described spirochete *Borrelia burgdorferi* was found to persist only in the midgut in the majority of ticks. Thus, from 4, to 5% of *I. dammini,* for instance, had a

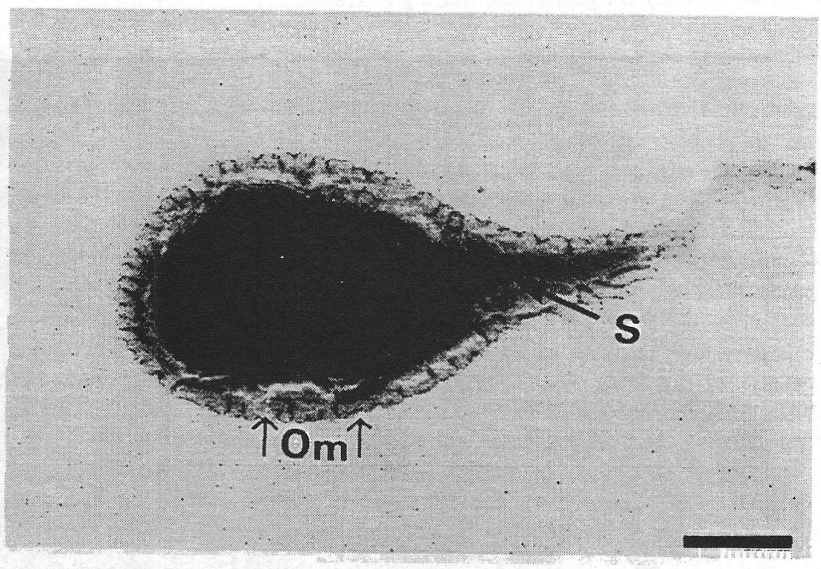

FIGURE 5.8. Negative staining of an encysted *Borrelia burgdorferi*. The spirochete folds and coils within portions of its outer membrane. S, terminus of spirochete; Om, outer membrane margin (bar, 0.2 μ).

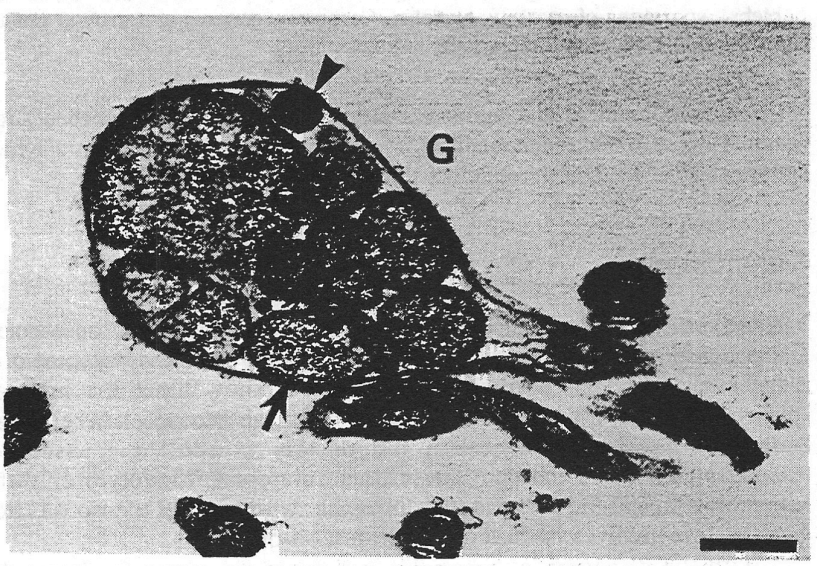

FIGURE 5.9. Cross-sectional profile of gamma containing an electron-dense granule (arrowhead) and several membrane-enclosed cytosolic masses (full arrow) (transmission electron micrograph; bar, 0.2 μ).

systemic infection with few spirochetes in the various tissues. Midgut penetration and subsequent tissue invasion are said to occur during feeding of infected ticks, and it is thought that transmission occurs via spirochete-containing saliva, although transmission via regurgitation has not as yet been ruled out.

Transovarial or vertical transmission, which is common to certain argasid tick–spirochete associations, has also been recorded for the Lyme disease borrelia, but it is rare and appears to be of no ecological significance. In fact, there is evidence that heavily infected oocytes fail to complete oogenesis.

Detection of *B. burgdorferi* in ticks not belonging to the *I. ricinus* complex suggests that other tick species might be involved in the natural history of this spirochete. Thus, there is strong evidence that the rabbit ticks *Haemaphysalis leporispalustris* and *I. dentatus* play a role in maintaining the Lyme disease spirochete among lagomorphs.

Although deerflies, horseflies, and mosquitoes are not considered vectors of *B. burgdorferi,* the presence of spirochetes in the digestive tract of these arthropods indicates they have ingested spirochetemic blood, and suggests the possibility of mechanical transmission.

Continued extensive field investigations into the ecology of *B. burgdorferi* in the United States and other countries will undoubtedly reveal not only additional vector associations of this agent but also new species of arthropod-borne borreliae.

Lastly, the development of spirochetes in their arthropod vectors and vertebrate hosts may indeed be more complex than assumed. The consistent finding of membrane-derived vesicles associated with *B. burgdorferi,* by fluorescence, scanning, and transmission electron microscopy, has rekindled interest in the nature of these vesicles, which in the past had been considered either to be degenerative products or germinative units in a complex developmental cycle. Molecular studies conducted so far have shown that these vesicles, called *blebs* and *gemmae,* contain genetic material in the form of linear and circular plasmids; they may be involved in the storage or the exchange of genetic material or both. The potential role of the chromatin bodies or granules in gemmae is the subject of ongoing research.

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