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Association of *Borrelia garinii* and *B. valaisiana* with Songbirds in Slovakia

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In Europe, 6 of the 11 genospecies of *Borrelia burgdorferi* sensu lato are prevalent in questing *Ixodes ricinus* ticks. In most parts of Central Europe, *B. afzelii*, *B. garinii*, and *B. valaisiana* are the most frequent species, whereas *B. burgdorferi* sensu stricto, *B. bissettii*, and *B. lusitaniae* are rare. Previously, it has been shown that *B. afzelii* is associated with European rodents. Therefore, the aim of this study was to identify reservoir hosts of *B. garinii* and *B. valaisiana* in Slovakia. Songbirds were captured in a woodland near Bratislava and investigated for engorged ticks. Questing *I. ricinus* ticks were collected in the same region. Both tick pools were analyzed for spirochete infections by PCR, followed by DNA-DNA hybridization and, for a subsample, by nucleotide sequencing. Three of the 17 captured songbird species were infested with spirochete-infected ticks. Spirochetes in ticks that had fed on birds were genotyped as *B. garinii* and *B. valaisiana*, whereas questing ticks were infected with *B. afzelii*, *B. garinii*, and *B. valaisiana*. Furthermore, identical *ospA* alleles of *B. garinii* were found in ticks that had fed on the birds and in questing ticks. The data show that songbirds are reservoir hosts of *B. garinii* and *B. valaisiana* but not of *B. afzelii*. This and previous studies confirm that *B. burgdorferi* sensu lato is host associated and that this bacterial species complex contains different ecotypes.

Lyme borreliosis is the most frequent arthropod-borne disease in humans living in moderate climates. Under the umbrella of the wider taxon *Borrelia burgdorferi* sensu lato, the bacteria constitute a group of 11 named genospecies, which were delineated based on DNA-DNA hybridization and sequence divergence of selected loci (2, 32, 34, 39, 40). In Europe, six genospecies are recorded as infecting *Ixodes ricinus* ticks (8, 15, 25), and the most prevalent genospecies are *B. afzelii*, *B. garinii*, and *B. valaisiana* (13, 25). In contrast to the case in northern America, *B. burgdorferi* sensu stricto and *B. bissettii* are relatively rare in Europe (8, 13, 15, 25). *B. lusitaniae* seems to be restricted to the western Mediterranean Basin, where its infection prevalence in ticks has been reported to be very high locally (3).

Since the delineation of genospecies in *B. burgdorferi* sensu lato, it has been discussed whether the species complex is differentiated ecologically (4, 17, 18, 26, 44). Our recent model of *B. burgdorferi* sensu lato transmission proposes that *B. burgdorferi* sensu lato comprises at least three major ecotypes which are associated with different sets of vertebrate host species (26, 28). Various testable predictions derived from this model have been validated in the field and in the laboratory. Field studies from Slovakia and Switzerland, for example, have shown that rodents preferentially transmit *B. afzelii* to ticks (13, 18). Furthermore, various studies indicate that some songbird species, seabirds, and pheasants are reservoir hosts of *B. garinii* and *B. valaisiana* (12, 17, 19, 27, 29). The transmission model also predicts that bird- and rodent-associated ecotypes of *B. burgdorferi* sensu lato segregate in individual questing ticks, a prediction that has also been validated (25).

The present work was carried out in a region of Europe where *B. afzelii*, *B. garinii*, and *B. valaisiana* are prevalent in questing ticks. In a previous study carried out in the same region, we have demonstrated that rodents are the reservoir hosts of *B. afzelii* but not of *B. garinii* and *B. valaisiana* (13). For this reason, hosts other than rodents must contribute to the prevalence of *B. garinii* and *B. valaisiana* in questing tick populations in this region. The major aim of this study, therefore, was to identify reservoir hosts of *B. valaisiana* and *B. garinii*, the agent of human neuroborreliosis, in Slovakia.

MATERIALS AND METHODS

Field sites and collection of questing ticks. The study was carried out in three localities within western Slovakia. Locality 1 (Podunajske Biskupice; 17°07′W, 48°08′N) is sylvatic and situated near the river Danube, 2 km southeast of Bratislava. The most prominent trees are *Quercus robur* (pedunculate oak), *Populus alba* (white poplar), and *Ulmus minor* (English elm). *Humulus lupulus* (hop), *Clematis vitalba* (traveler's joy), and *Solidago gigantea* (giant goldenrod) cover most parts of the ground. Locality 2 (Malacky; 17°08′W, 48°30′N) is a sylvatic park in the middle of the town Malacky, 35 km northwest or Bratislava. In this habitat *Acer campestre* (field maple), *Carpinus betulus* (hornbeam), *Crateagus monogyna* (hawthorn), *P. alba*, *Prunus padus* (European birdcherry), *Tilia cordata* (small-leaved lime), and *Sambucus nigra* (elder) predominate. Lo-

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TABLE 1. *B. burgdorferi* sensu lato infections in questing *I. ricinus* ticks

Locality (yr)	Stage	No. of ticks tested	Number (%) of ticks infected with[a]:											
			BBU sensu lato	BBU	BGA	BVA	BAF	BLU	BGA, BAF	BGA, BVA	BBU, BAF	BVA, BAF	BAF, BGA, BVA	BAF, BGA, BBI
Podunajské Biskupice (1999)	Nymphs	130	28 (21.5)			2	25			1				
	Adults	239	76 (31.8)	1	21	8	40	2		4				
Malacky (1999)	Nymphs	198	37 (18.7)		8	7	16			3		2		1
	Adults	147	59 (40.1)	1	12	12	29		1	2	2			
Záhorská Ves (1999)	Nymphs	54	15 (27.8)	1	3	2	9							
	Adults	198	96 (48.5)	2	5	8	59	4	1	16			1	
Záhorská Ves (2000)	Nymphs	174	33 (19.0)		2	15	16							
	Adults	154	76 (49.4)		6	38	29	3						
Total		1,294	420 (32.5)	5	57	92	223	9	2	26	2	2	1	1

[a] BBU sensu lato, *B. burgdorferi* sensu lato; BBU, *B. burgdorferi* sensu stricto; BGA, *B. garinii;* BVA, *B. valasiana;* BAF, *B. afzelii;* BLU, *B. lusitaniae;* BBI, *B. bissettii.*

cality 3 (Zahorska Ves; 16°53′W, 48°22′N) is a mixed deciduous and coniferous woodland situated at alluvial sands close to the river Morava, 45 km northwest of Bratislava. The tree layer consists mainly of *Q. robur*, *Pinus sylvestris* (scotch pine), and *Robinia pseudoacacia* (false acacia). The undergrowth is dominated by *S. nigra*, *Ligustrum vulgare* (European privet), *Calamagrostis epigeios* (wood smallreed), *C. monogyna*, *Dactylus glomerata* (orchard grass), and *R. pseudoacacia*.

Within each locality, four sampling sites (each measuring 100 m^2) were selected. Questing nymphal and adult ticks of *I. ricinus* were collected from the vegetation by blanket dragging at regular 2-week intervals in the spring and early summer of 1999. During 2000, field work was conducted only in locality 3 (Zahorska Ves) every 2 weeks from March until November. Field-derived ticks were preserved in 70% ethanol.

Capture of birds. From March through June 2001, birds were mist-netted at a sylvatic locality near Jursky Sur (17°13′W, 48°15′N). The locality is situated 12 km northeast of Bratislava. The tree layer is formed by *A. campestre*, *T. cordata*, *T. platyphylla* (large-leaved lime), and *Q. robur*. The undergrowth is dense, consisting of *S. nigra*, *Prunus spinosa* (blackthorn), *C. monogyna*, *Rubus caesius* (dewberry), and *L. vulgare*. Netted birds were carefully removed, identified to species, weight, measured, identified as to sex, and examined for ticks. Feeding ticks were taken and preserved in 70% ethanol. Thereafter, all birds were released.

Detection of *B. burgdorferi* sensu lato in ticks. *I. ricinus* ticks were examined for *B. burgdorferi* sensu lato genospecies by PCR, followed by the reverse line blot assay and, for a subsample, by nucleotide sequencing. Genomic DNA was extracted from ticks by alkaline hydrolysis (10). The nested PCR targeted the *rrf* (5S)-*rrl* (23S) intergenic spacer as described previously (13, 27, 45). To prevent DNA contamination, all stages of the PCR were carried out under strictly aseptic conditions with PCR hoods, and they were also separated temporally and spatially. In addition, negative controls at a ratio of 2:3 were included. As positive controls, serial dilutions of cultured *B. andersonii*, a genospecies confined to America, were used. The PCR products were hybridized to DNA probes specific for *B. burgdorferi* sensu lato, *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, and *B. valaisiana* as described previously (27, 45). In this study, a *B. bissettii*-specific probe was designed whose oligonucleotide sequence (5′-aminolink spacer-ATA TAAAATTTAGAACTAAAATAAAATAC) is complementary to nucleotide positions 28 to 56 of the 5S-23S intergenic spacer region. Amplicons derived from cultured strains (*B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, *B. valaisiana*, and *B. bissettii*) were used to confirm the specificities of the DNA probes.

DNA sequencing of PCR products. Samples that could not be genotyped by the reverse line blot assay as well as randomly selected *B. garinii* samples were amplified at the *ospA* locus, and their nucleotide sequences were determined. Sequence analysis was carried out with the Lasergene99 system (DNASTAR, Inc.). Unique sequences have been deposited in GenBank.

Statistical analysis. Differences in the infection prevalence of *B. burgdorferi* sensu lato genospecies between localities and developmental stages of questing ticks were analyzed by means of weighted logistical regression analysis (i.e., with binomial errors and weighted according to sample size).

Nucleotide sequence accession numbers. The GenBank accession numbers for *B. garinii* alleles 4, 5, and 6 (see Tables 5 and 6) are AY226824, AY226822, and AY226823, respectively.

RESULTS

Questing ticks. A total of 1,294 questing *I. ricinus* ticks were tested for infection with *B. burgdorferi* sensu lato by PCR. Of these, 420 (33%) were infected with *B. burgdorferi* sensu lato (Table 1). Six genospecies of *B. burgdorferi* sensu lato were identified, with 34 ticks being infected with more than one genospecies. Considering the presence of multiple infections in individual ticks, the number of spirochete infections in the data set was calculated ($n = 456$). For example, an individual tick infected with three different genospecies was regarded as equivalent to three infections. The most frequent of all genospecies was *B. afzelii* (50.6%), followed by *B. valasiana* (26.5%) and *B. garinii* (19.1%). These three genospecies constituted the vast majority of spirochete infections (96.2%), whereas *B. burgdorferi* sensu stricto, *B. bissettii*, and *B. lusitaniae* were very rare in the data set. The most frequent genospecies combination in individual ticks with mixed infections was *B. garinii* and *B. valaisiana* (27 of 34). In contrast, only few ticks harboring *B. afzelii* were concurrently infected with *B. garinii* (4 of 34) or *B. valaisiana* (3 of 34) (Table 1).

Significantly more adult ticks than nymphs were infected with *B. burgdorferi* sensu lato ($\chi^2 = 29.05$; df = 1; $P = 7.053 \times 10^{-8}$) (Table 1). *B. afzelii* contributed to 57% of the infections in nymphs and to 48% of those in adult ticks. Taken together, *B. garinii* and *B. valaisiana* contributed to 41% of the infections in nymphs and to 47% of those in adult ticks. The infection prevalences of *B. burgdorferi* sensu lato and of individual genospecies were compared between developmental tick stage and locality. First, *B. burgdorferi* sensu lato was most prevalent in locality 3 ($\chi^2 = 11.49$; df = 1; $P = 7.00 \times 10^{-4}$). Second, the major three genospecies in the data set, *B. afzelii*, *B. garinii*, and *B. valaisiana*, were found in all three localities. For *B. afzelii*, a higher infection prevalence was found in adult ticks than in nymphs ($\chi^2 = 5.812$; df = 1; $P = 0.0159$), and it was most prevalent in locality 3 ($\chi^2 = 11.18$; df = 1; $P = 8.27 \times 10^{-4}$).

TABLE 2. List of birds not carrying ticks or *B. burgdorferi* sensu lato-infected ticks

Bird species	No. of birds	No. of ticks					
		Tick infestation		Tested ticks		Positive ticks	
		Larvae	Nymphs	Larvae	Nymphs	Larvae	Nymphs
Aegithalos caudatus (Linnaeus, 1758) (long-tailed tit)	2	0	0				
Coccothraustes coccothraustes (Linnaeus, 1758) (hawfinch)	1	0	0				
Dendrocopos major (Linnaeus, 1758) (great spotted woodpecker)	4	0	0				
Muscicapa striata Pallas, 1764 (spotted flycatcher)	4	0	0				
Passer montanus (Linnaeus, 1758) (tree sparrow)	2	0	0				
Phylloscopus collybita (Vieillot, 1819) (chiffchaff)	1	0	0				
Parus caeruleus (Linnaeus, 1758) (blue tit)	2	0	3	0	3	0	0
Erithacus rubecula (Linnaeus, 1758) (European robin)	10	63	5	25	3	0	0
Fringilla coelebs (Linnaeus, 1758) (chaffinch)	4	0	2	0	2	0	0
Luscinia megarhynchos (Brehm, 1831) (nightingale)	1	1	0	0	0	0	0
Parus montanus (Baldenstein, 1827) (willow tit)	1	3	4	3	4	0	0
Parus palustris (Linnaeus, 1758) (marsh tit)	1	0	1	0	1	0	0
Passer domesticus (Linnaeus, 1758) (house sparrow)	1	1	0	1	0	0	0
Sylvia atricapilla (Linnaeus, 1758) (blackcap)	12	3	6	3	5	0	0

The infection prevalence of *B. garinii* was also higher in adult ticks than in nymphs ($\chi^2 = 13.2$; df = 1; $P = 2.80 \times 10^{-4}$); however, no significant differences between localities were observed ($\chi^2 = 1.858$; df = 2; $P = 0.395$). For *B. valaisiana*, the infection prevalence was higher in adult ticks than in nymphs ($\chi^2 = 6.42$; df = 1; $P = 0.0113$), and it was more prevalent in localities 2 and 3 than in locality 1 ($\chi^2 = 9.82$; df = 1; $P = 0.00173$).

In locality 3, questing ticks were also collected in March to November 2000, thus covering spring, summer, and autumn. Since both the structure of the host community and the activity of *I. ricinus* are known to vary with season, we were interested to see whether the infection prevalences of *B. burgdorferi* sensu lato and of the individual genospecies would also vary with season. However, no differences in the infection prevalence of any genospecies between the period of March to June and July to November 2000 was observed ($\chi^2 = 0.139$; df = 1; $P = 0.709$). Furthermore, no difference in the overall infection prevalence of *B. burgdorferi* sensu lato between the years 1999 and 2000 was found ($\chi^2 = 0.2907$; df = 1; $P = 0.590$). For *B. burgdorferi* sensu stricto, *B. bissettii*, and *B. lusitaniae*, no significant site-, stage-, or year-specific trend was determined, due to insufficient data.

Together, the data show that *B. afzelii*, *B. garinii*, and *B. valaisiana* are the most frequent genospecies in questing ticks in this region of Central Europe. About half of the infections in questing ticks were caused by *B. garinii* and *B. valaisiana*, and the other half were caused by *B. afzelii*.

Ticks feeding on birds. Sixty-two birds of 17 bird species were captured in 2001. Of these, 14 species were either not infested with ticks or were infested with ticks that were found to be spirochete free (Table 2). Three species, the great tit (*Parus major*), the song thrush (*Turdus philomelos*), and the blackbird (*T. merula*), were infested with spirochete-infected ticks. Therefore, this paper focuses on these three songbird species.

Sixteen individual birds of these three songbird species were captured, and 69 engorged ticks were counted. The birds were more heavily infested with nymphs than with larvae. Of the 57 feeding ticks tested, 16 (28%) were infected with *B. burgdorferi* sensu lato. Genotyping revealed that feeding larvae and nymphs were infected with *B. garinii* (11 of 16) and *B. valaisiana* (6 of 16). Despite the high relative prevalence in questing nymphs, no *B. afzelii* infection in larvae and nymphs engorged on the birds was detected (Tables 3 and 4).

In order to fingerprint the *B. garinii* strains found in questing ticks and in ticks engorged on the birds, the *ospA* genes of randomly selected samples derived from both tick pools were amplified and sequenced. Among the 11 *ospA* sequences analyzed from questing ticks, five different alleles were identified. Three of these *B. garinii* alleles were identical to *ospA* sequences specific for previously characterized reference strains,

TABLE 3. *B. burgdorferi* sensu lato infections in *I. ricinus* ticks feeding on songbirds

Bird species	No. of examined birds	No. of tested ticks[a]		Number of ticks infected with:							
				B. burgdorferi sensu lato		*B. garinii*		*B. valaisiana*		*B. garinii* and *B. valaisiana*	
		L	N	L	N	L	N	L	N	L	N
P. major (Linnaeus, 1758)	11	2	11	1	2	1	1		1		
T. merula (Brehm, 1831)	2	2	12	1	3		1	1	2		
T. philomelos (Linnaeus, 1758)	3	12	18	3	6	2	5	1			1
Total	16	16	41	5	11	3	7	2	3		1

[a] L, larvae; N, nymphs.

TABLE 4. Infection prevalence of *B. garinii*, *B. valaisiana*, and *B. afzelii* in questing ticks and ticks feeding on birds

Genospecies	Infection prevalence (%) in:			
	Questing ticks		Feeding ticks	
	Nymphs	Adults	Larvae	Nymphs
B. garinii	3.2	9.3	18.7	19.5
B. valaisiana	5.7	12.1	12.5	9.7
B. afzelii	12.4	22.0	0.0	0.0

whereas two alleles have so far not been described (Table 5). Among the eight *ospA* sequences obtained from ticks that had fed on songbirds, four alleles were found, three of which were also present in the questing tick pool. One allele was unique. None of the *ospA* alleles indicated infections with OspA serotype 4 or NT 29-like strains (Table 6).

DISCUSSION

The present study confirms that songbirds are reservoir hosts of *B. garinii* and *B. valaisiana* in Central Europe. The localities studied represent typical forest habitats of Central Europe. The overall infection prevalence of *B. burgdorferi* sensu lato of 33% in questing *I. ricinus* is consistent with previous findings (13, 25). Six genospecies were detected in questing ticks, the vast majority of which were identified as *B. afzelii*, *B. valaisiana*, and *B. garinii*. With some variation in prevalence, these three genospecies were present in all of the localities investigated in this study. They have also been found to be the most prevalent genospecies in other regions from Central Europe (8, 15, 18). The prevalences of *B. burgdorferi* sensu stricto, *B. bissettii*, and *B. lusitaniae* in questing ticks were very low, a finding that appears to be typical for Central Europe (15). In the western Mediterranean Basin, however, high prevalences of *B. lusitaniae* have been observed in some localities, suggesting that this genospecies has a narrow spectrum of reservoir hosts, restricting its geographical range (3).

In a recent study carried out in the same region as this study, we have shown that rodents, such as mice and voles, are reservoir hosts of *B. afzelii* but not of *B. garinii* or *B. valaisiana* (13). Thus, *B. garinii* and *B. valaisiana* must be maintained by tick hosts other than rodents. As emerging evidence from previous studies had pointed to ground-foraging birds as the prime candidates (12, 17, 27, 38), the present study focused on the analysis of songbirds. Three of the 17 captured songbird species were infested with spirochete-infected larvae and nymphs of *I. ricinus*. All infections were genotyped as *B. garinii* and *B. valaisiana*, showing that these songbirds are reservoir competent for these genospecies. Given the limited number of larvae engorged on the birds tested in this study, precise quantitative estimates of spirochete infectivity (35) of these birds cannot yet be provided. However, a combined infection prevalence of *B. garinii* and *B. valaisiana* of about 30% in larval ticks that had fed on these birds indicates that these three songbird species are as efficient in transmitting these two genospecies to ticks as European rodent populations are for *B. afzelii* (13, 18). Eight of the bird species captured in the present study were parasitized with spirochete-free *I. ricinus* ticks. The reasons for this finding are not known. Mathematical models of Lyme borreliosis will have to estimate the force of infection for each genospecies or even OspA serotype (42, 43).

B. garinii is highly polymorphic and comprises six OspA serotypes and different ribotypes (6, 14, 33). Recent evidence indicates that some Asian strains of *B. garinii* thrive in rodent hosts rather than in avian hosts (34, 36). Furthermore, recent studies suggest that OspA serotype 4 strains are associated with rodents rather than with birds (14, 16). Therefore, it was necessary to investigate whether the avian hosts analyzed in this study transmit the same *B. garinii* strains to feeding ticks as those prevalent in the questing tick pool. None of the sequenced *B. garinii* strains represented OspA serotype 4 strains or the NT 29-like strains. Three of the six *ospA* alleles found in the data set were shared by the questing tick pool and the tick pool engorged on the birds. Allele 3 is identical to the *ospA* sequence of reference strain ZQ1ox/Rio2 (6), and we have shown experimentally and epidemiologically that the pheasant is also reservoir competent for this strain of *B. garinii* (27, 29). Furthermore, this strain of *B. garinii* displays full resistance against avian complement but is readily lysed by complement of rodents and larger mammals, such as deer, cows, and sheep (30), a property also observed for the strains VSBP and VSBM (K. Kurtenbach, unpublished observation). Blackbirds have also been found to be reservoir hosts of *B. garinii* in Switzerland (7, 17). Therefore, it can be concluded that songbirds and other bird species, such as the pheasant (27), are important reservoir hosts of the vast majority of *B. garinii* and *B. valaisiana* strains in questing ticks in terrestrial habitats of Europe (19).

Of the questing nymphs collected in this study, 12% were infected with *B. afzelii.* Therefore, songbirds must encounter *B. afzelii*-infected ticks. The lack of *B. afzelii* amplification in ticks feeding on the birds indicates that songbirds are not transmission competent for this genospecies. A similar finding has recently been made experimentally with the pheasant as avian model of Lyme borreliosis (29). That study also showed that

TABLE 5. *B. garinii ospA* alleles in questing ticks

Allele	No. of samples	Reference strain (% similarity)	OspA serotype of references strain	Sensitivity or resistance to complement
1	1	VSBP (100)	6	Sensitive to rodent, resistant to pheasant
2	2	VSBM (100)	5	Sensitive to rodent, resistant to pheasant
3	4	ZQ1ox (100)[a]	3	Sensitive to rodent, resistant to pheasant
4	2	PBr (97)	3	ND[b]
5	2	T25 (94)	7	ND

[a] ZQ1ox is described in reference 29, and its *ospA* sequence is identical to that of the Rio2 strain (6).
[b] ND, not determined.

TABLE 6. *B. garinii ospA* alleles in ticks engorged on songbirds

Allele	No. of samples	Reference strain (% similarity)	OspA serotype of reference strain	Sensitivity or resistance to complement
1	1	VSBP (100)	6	Sensitive to rodent, resistant to pheasant
3	1	ZQ1ox (100)[a]	3	Sensitive to rodent, resistant to pheasant
5	5	T25 (94)	7	ND[b]
6	1	T25 (99.4)	7	ND

[a] ZQ1ox is described in reference 29, and its *ospA* sequence is identical to that of the Rio2 strain (6).
[b] ND, not determined.

this genospecies was eliminated in preinfected ticks that had fed on the birds. Most interestingly, a reverse pattern of spirochete survival in engorged ticks was found for rodents captured in Slovakia (13). Not only did the rodents fail to infect tick larvae with *B. garinii* and *B. valaisiana*, but these two genospecies were also eliminated from nymphs feeding on the rodents.

As opposed to rodents (1), birds are highly mobile, and some songbird species are long-distance migrants (11, 19, 38, 41, 44). Migratory restlessness reactivates spirochete infection, which may enhance transmission (11). Therefore, it is likely that avian migration has a major impact on the population structure of *B. burgdorferi* sensu lato, as bird-associated spirochetes are likely to be spread over large distances within a time frame too short to allow for the accumulation of mutations. In fact, the *ospA* gene of the *B. garinii* strain, ZQ1ox, found in ticks engorged on the songbirds from Slovakia is identical to that of spirochetes detected in Spain (6), France (unpublished data), and the United Kingdom (6, 29).

The concept of host association of *B. burgdorferi* sensu lato is now firmly based on empirical evidence and can therefore be regarded as a scientific fact (26). The host association is determined by the interaction of spirochetes with host complement in an antibody-independent manner (23, 28, 30, 31, 37). *B. burgdorferi* sensu lato strains that are associated with a particular host species are always resistant to the alternative pathway of complement of this host. In contrast, sera from hosts that are reservoir incompetent for a *B. burgdorferi* sensu lato strain often lyse those strains (26, 28, 47). The patterns of differential survival of the *B. burgdorferi* sensu lato genospecies in ticks engorged on rodents and birds suggest that complement operates in the gut of the feeding tick (5, 13, 26, 29). Resistance to complement is now known to be mediated by the binding of host-derived complement control proteins, such as factor H (20–22). OspE and related proteins (Erps) which are expressed by spirochetes residing in the tick gut (9) have recently been identified as ligands of factor H (46). It has, therefore, been suggested that the particular *erp* gene repertoire of a *B. burgdorferi* sensu lato cell determines its ability to resist complement-mediated lysis, thereby defining its ecotype (26, 28, 46). The *erp* genes constitute a superfamily of plasmid-borne, multigene families. These represent phage genomes for which transduction as a mode of lateral gene transfer has been demonstrated (46). This mechanism of genetic recombination may in part explain adaptive radiation of *B. burgdorferi* sensu lato.

It is now apparent that the species complex of *B. burgdorferi* sensu lato comprises at least three ecotypes: (i) a rodent-associated ecotype, (ii) a bird-associated ecotype, and (iii) an ecotype that thrives in both rodent and avian hosts (24, 26, 28, 44). In Europe, bird-associated and rodent-associated *B. burgdorferi* sensu lato strains circulate in distinct transmission cycles involving mainly birds and rodents and one tick species, *I. ricinus*. In conclusion, complement-mediated selection of *B. burgdorferi* sensu lato appears to be a major factor in the evolution and ecology of Lyme borreliosis spirochetes.

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