

## SOME PECULIARITIES OF THE MATING BEHAVIOR IN *IXODES PERSULCATUS* AND *IXODES RICINUS* TICKS (ACARINA, IXODIDAE): DIFFERENCES IN A SEXUAL TRANSMISSION OF THE SPECIES OF *BORELLIA*

### НЕКОТОРЫЕ ОСОБЕННОСТИ ПОЛОВОГО ПОВЕДЕНИЯ *IXODES PERSULCATUS* И *IXODES RICINUS* (ACARINA, IXODIDAE): РАЗЛИЧИЯ В ПОЛОВОЙ ПЕРЕДАЧЕ РАЗНЫХ ВИДОВ БОРРЕЛИЙ

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#### ABSTRACT

The mating behavior is demonstrated to be different in *Ixodes ricinus* L. and *I. persulcatus* Schulze. In *I. ricinus* the male and female are found to prefer to copulate mainly on the horizontal surface whereas in *I. persulcatus* they copulate most often on the vertical one. Also in *I. persulcatus* the copulation time is shown to be shorter when the copulation takes place on the vertical surface. The coupling *per se* is achieved in a shorter time when performed on the vertical surface. The infestation of one or both partner ticks with *Borrelia* increases the copulation time in *I. persulcatus*. *I. ricinus* ticks, heavily infested with *Borrelia afzelii*, refrain from coupling. The locomotor activity of coupling pairs of *I. persulcatus* is less than in non-infested ones. Ticks, which were kept in pairs, are found to be infested in a larger scale than those being kept alone. The transmission of *Borrelia garinii* in the taiga tick, *I. persulcatus*, from males to females is recorded. In 4.5% cases *B. garinii* were identified in unengorged larvae of *I. persulcatus* collected in the field. Preliminary data suggest that namely *B. afzelii* is transmitted from the male to female in *I. ricinus*.

#### РЕЗЮМЕ

Показано, что поведение *Ixodes ricinus* L. и *Ixodes persulcatus* Schulze, копулирующих вне хозяина, различается. Особи *I. ricinus* в большинстве случаев копулируют на горизонтальной поверхности, тогда как *I. persulcatus* — на вертикальной. Время копуляции *I. persulcatus* на вертикальной поверхности меньше, чем на горизонтальной, и копуляция наступает раньше, чем на горизонтальной поверхности. Зараженность боррелиями одного или двух

половых партнеров увеличивает время копуляции *I. persulcatus*. *I. ricinus*, более интенсивно зараженные *Borrelia afzelii*, вообще не копулируют. Активность зараженных боррелиями копулирующих пар *I. persulcatus* меньше, чем у незараженных. Клещи *I. persulcatus*, содержащиеся в парах, были заражены в большем проценте случаев, нежели клещи, содержащиеся поодиночке. Установлена передача *Borrelia garinii* от самцов самкам таежного клеща *I. persulcatus*. Обнаружен возбудитель нейроборрелиоза *B. garinii* в голодных личинках *I. persulcatus* (в 4.5% случаев). По предварительным данным, самцы *I. ricinus* передают самкам преимущественно *B. afzelii*.

#### INTRODUCTION

Sexual behavior of ticks, which copulate on vertebrate hosts, was studied extensively [Feldman-Muhsam, Borut, 1971; Oliver et al., 1974]. Chemicals, which determine the meeting of females and males of those ticks, are known [Sonenshine et al., 1982] and have even been used for the control of some Ambliomminae [Sonenshine et al., 1998]. However, the mating behavior of ticks of the genus *Ixodes*, including *Ixodes persulcatus* Schulze and *I. ricinus* (L.), and the factors, which determine their meeting on the ground or on vegetation, are still not known. Babenko [1985] stated that 100% of *I. ricinus* and near 50% of *I. persulcatus* collected by flagging were inseminated. Guerin et al. [1999] demonstrated that both sexes of *I. ricinus* were attracted by the guanine odor. Balashov [1998] obtained data that faeces of moulted adult ticks consist of the guanine only, which might attract sexes to each

other. Bouman et al. [1999] proved that the moist air saturated by odor of unengorged *I. ricinus* females, collected in the field, which has been pumped through the Y-form or 4-angled olfactometer, attracted *I. ricinus* males to females. In Y-form olfactometer males were attracted in 70.9 of cases and in 4-angled one in 62.5% of cases.

Another factor that might be important for the behavior of eyeless *Ixodes* ticks is their exposition to various plant odors. According to Alekseev et al. [1991] the preference of some plants for *I. persulcatus* females to crawl up [Pomerantsev, Serdyukova, 1948] is based on their different reactions to plant odors. It was stressed that attractant or deterrent action of plant odors may be dependent not only on the tick sex but also on the tick infection rate with the tick-borne encephalitis virus (TBEV). Knowledge of the sexual behavior in *Ixodes* is practically important because of the earlier proved transovarial transmission of this pathogen by the females of *I. persulcatus* inseminated by TBEV infected males to 10% of their progeny [Chunikhin et al., 1983]. According to Balashov and Grigoryeva [1998] 77 out of 77 (100%) unfed *I. persulcatus* larvae collected in the field in 1997 were infected with *Borrelia burgdorferi sensu lato*. These data seem to be dubious because only 1 out of 22 (4.5%) of *I. persulcatus* larvae collected in St. Petersburg vicinities were infected with *B. garinii*. Our results are supported by data obtained by Rijpkema et al. [1994] which found *B. afzelii* only in 5% of studied larvae of *I. ricinus*. It is of interest to know in what way the mating behavior determines the differences in the insemination rates of *I. ricinus* (100%) and *I. persulcatus* (50%) collected from the ground [Babenko, 1985]. It was proposed that *I. ricinus* copulate mainly on the ground whereas *I. persulcatus* mate either on vegetation (possibly being attracted by plant odors), or on the host animal. The attractiveness of ticks to animal host odors before or immediately after the tick is attached to the host skin was earlier shown by Naumov [1996] and Leonovich [1999]. We attempt to elucidate the potential and species-specific differences in *Borrelia* transmission by two above-mentioned species of ticks.

#### MATERIALS AND METHODS

*Ixodes persulcatus* ticks were collected by flagging in the vicinities of St. Petersburg during 1997–1999 field seasons. *I. ricinus* were collected at spring and fall peaks or their activity on the Curonian Spit (Kaliningrad enclave) in 1997–1998. Males and females were maintained in couples in the glass tubes with a various relative humidity. The tubes were placed either horizontally or vertically. Both the time needed to find a

mate and the longevity of copulation were checked. Some male and female ticks were reared in individual tubes. Parameters of the tick activity (speed, height, total activity) were estimated for all tested ticks in accordance with the methods earlier suggested by us with the application of the inclined “tickdrome” [Alekseev, 1996; Alekseev et al., 1996, 2000]. Upon the completion of the experiment all specimens of ticks were dissected, and *Borrelia* presence and number were determined using the dark-field microscopy (10x40). *Borrelia* positive ticks were investigated using PCR and RLB methods [Rijpkema et al., 1995, 1996; Schouls et al., 1999] to identify the pathogen’s species.

128 couples were studied in the experiments to observe the differences in the copulation behavior depending on which surface, horizontal or vertical one, was being offered to ticks. Same ticks were used for the evaluation of the activity parameters. Sexual transmission of spirochetes was confirmed on the basis of the infection rate of 190 couples and 302 single reared *I. persulcatus* ticks. 113 couples of *I. persulcatus* were studied to obtain the data on the preferential transmission of different species of *Borrelia*. *t* and *chi-square* tests were used in the data analysis [Wilkinson, 1990].

#### RESULTS

Our experiments suggest that *I. ricinus* ticks prefer the horizontal surface for their mating versus the vertical one ( $p < 0.01$ ) (Fig. 1, A). In *I. persulcatus* partners are more often copulate on the vertical surface. Despite the surface type preference for *I. persulcatus* was not statistically significant (Fig. 1, B), it is important to stress that *I. persulcatus* ticks copulation on the vertical surface was observed 6.6 times more often than that of *I. ricinus*. Precopulation time was very similar in both species (25–50 minutes) irrespective of the type of the surface on which the copulation took place (Fig. 2, A, B). In *I. persulcatus* the copulation time was 1.5 times shorter on vertical surface comparing to horizontal one and 1.3–1.5 times shorter than the copulation time of *I. ricinus* ticks on both surfaces. The longest copulation time in *I. persulcatus*, recorded in the laboratory conditions, was 328 min for the vertical surface and 660 min for the horizontal one. The determination of spirochete infection rate among the couples, which were copulated, showed the prevalence value of 26.2% for *I. persulcatus*, and 14.5% for *I. ricinus*. The difference in the copulation time between uninfected mates of *I. persulcatus* and those infected with *Borrelia* was not statistically significant (Fig. 3). Nevertheless the mean copulation time increased when at least one sexual partner was infected or when the mean number of

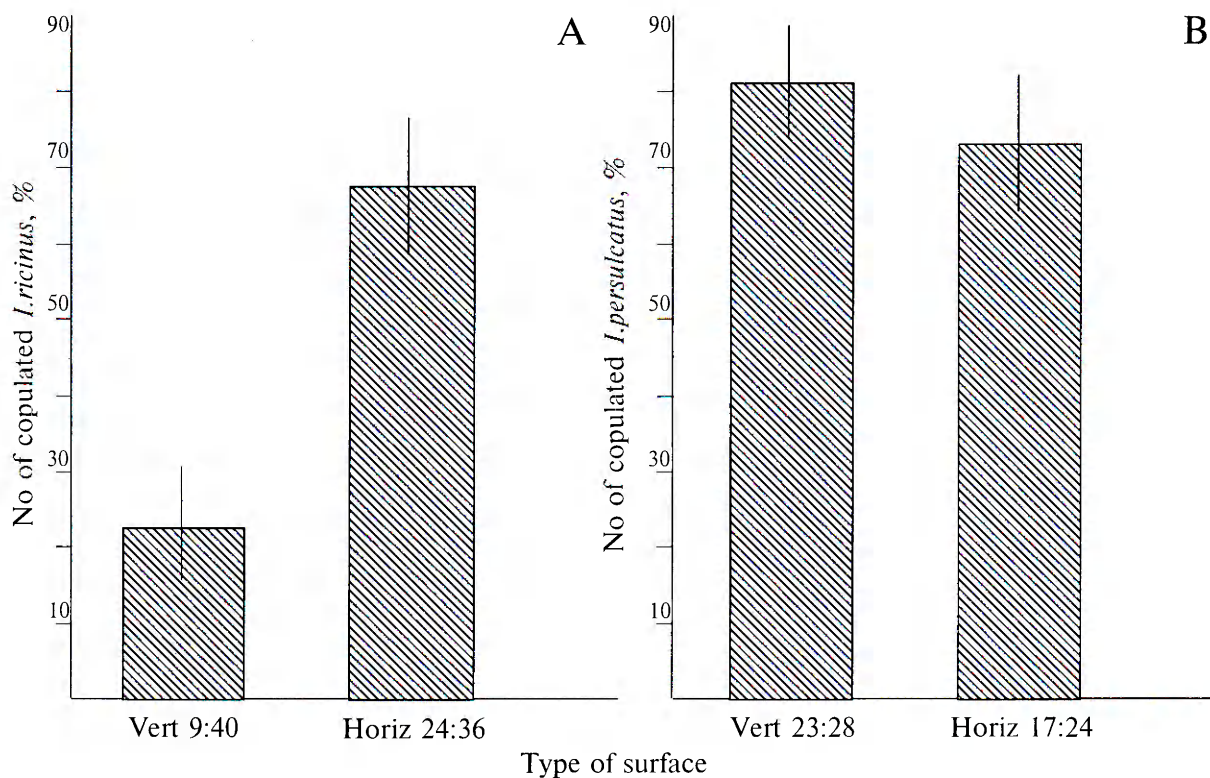


Fig. 1. The comparative ability of the ticks *Ixodes ricinus* (A) and *I. persulcatus* (B) to copulate on the vertical and horizontal surfaces.

Рис. 1. Способность к копуляции *Ixodes ricinus* (A) и *I. persulcatus* (B) на вертикальной и горизонтальной поверхностях.

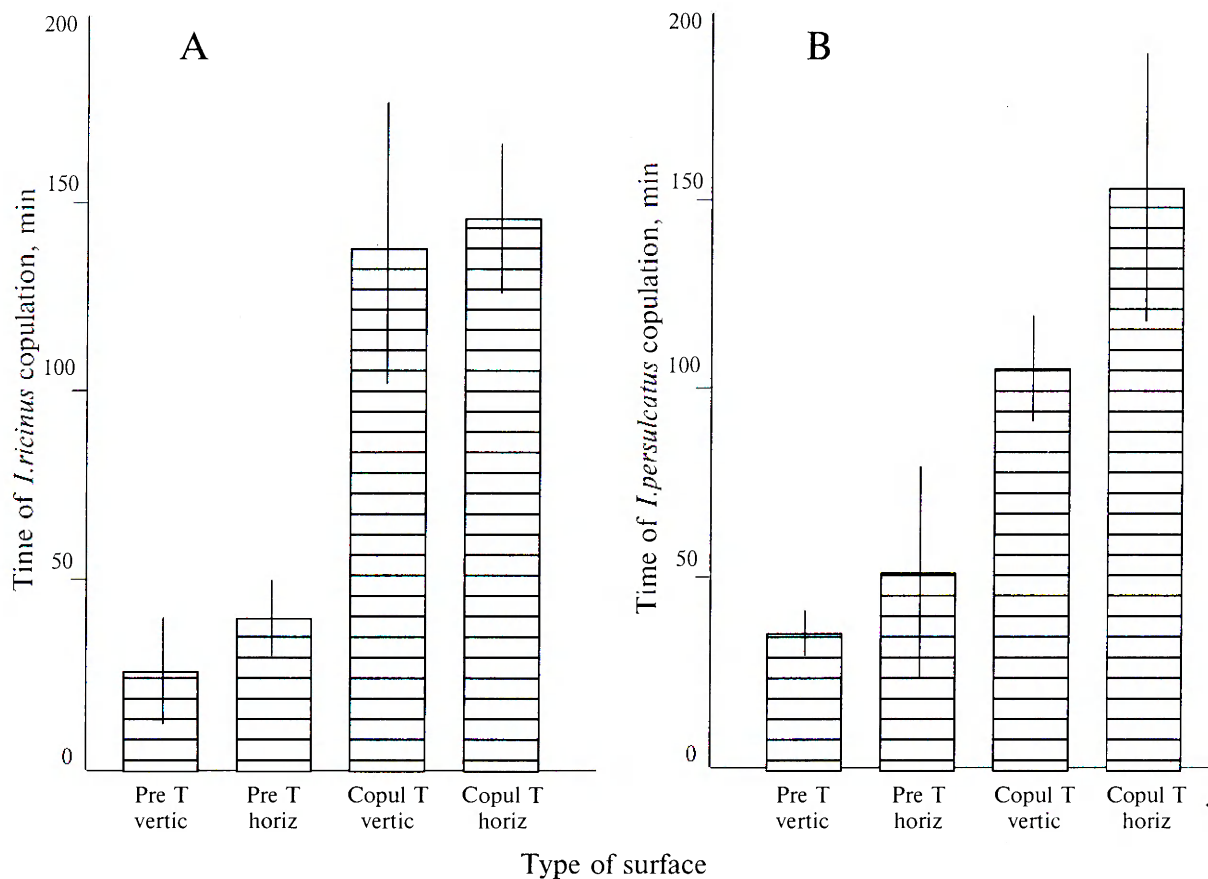


Fig. 2. Precopulation and copulation time of *Ixodes ricinus* (A) and *I. persulcatus* (B) on the vertical and horizontal surfaces.

Рис. 2. Время, предшествующее копуляции, и продолжительность копуляции *Ixodes ricinus* (A) и *I. persulcatus* (B) на вертикальной и горизонтальной поверхностях.



Table 1  
*Borrelia burgdorferi* sensu lato prevalence in ticks *Ixodes persulcatus* maintained in couples or in singles in the humidity gradient glass tubes (1992 and 1995 seasons).

Таблица 1  
 Зараженность *Ixodes persulcatus* *Borrelia burgdorferi* sensu lato при содержании клещей в пробирках дифференцированной влажности поодиночке и в парах (наблюдения 1992 и 1995 гг.).

Sex	Type of tick maintenance				Statistical significance	
	separate		in couples		td	p
	abs.	%	abs.	%		
Male	21/142	14.8±2.97	60/190	31.58±3.4	3.72	<0.01
Female	29/161	18.0±3.01	59/190	31.05±3.36	2.9	<0.01

Table 2  
 A preferential *Borrelia garinii* transmission by *Ixodes persulcatus* males to females [Alekseev et al., 1999].

Таблица 2  
 Избирательная передача боррелий вида *Borrelia garinii* самцами *Ixodes persulcatus* [Alekseev et al., 1999].

<i>Borrelia</i> species in donor tick	Number of infected partners in couples tested	
	Male ticks infected (n=24)	Female ticks infected (n=45)
	Infected females/Infected males	Infected males/Infected females
<i>B. afzelii</i>	0/7	0/9
<i>B. garinii</i>	4/9	0/24, p<0.01
<i>B. afzelii</i> + <i>B. garinii</i>	3*/8	0/12, p<0.05

\*In 2 female ticks was detected only *B.garinii*, 1 female tick carried a dual infection.

spirochetes in their bodies increased. Heavily infected *I. ricinus* refrained from copulation on either surface (Fig. 4).

Measuring the activity parameters of the naive ticks demonstrated that the movement speed as well as the entire activity decreased during the copulation (Fig. 5, A), whereas the height of their ascending increased. The speed, the height of ascending and the entire activity of the infected specimens in *copula* and *post copula* phases decreased in comparison with the same parameters of ticks recorded before the copulation (Fig. 5, B).

The prevalence of *Borrelia*-infected *I. persulcatus* was 1.5–2 times higher when ticks were maintained in couples comparative to singles (Table 1) [Alekseev, Dubinina, 1996]. In *I. persulcatus* the males were shown to transmit *B. garinii* to their mates. However the females were not able to infect their sexual partners (Table 2). The sexual transmission of *B. afzelii* from the male to female *I. persulcatus* was not recorded when only males were infected by this pathogen. *B. garinii* was detected only in one case among the larvae of *I. persulcatus* collected in the field and later tested

by PCR (1 of 22, or 4.5%), whereas *B. afzelii* were never obtained from the larvae of this species. The preliminary analysis of the *Borrelia* species prevalence in *I. ricinus* maintained in couples (20) suggests that the males of this species transmit *B. afzelii* mainly to their mates.

## CONCLUSION

Our data demonstrated the presence of differences in the mating behavior between representatives of the sheep (forest) tick (*I. ricinus*) and the taiga tick (*I. persulcatus*). The former species, which is phylogenetically close to the nidicolous tick, *Ixodes hexagonus* [Black, Piesman, 1994], was shown to copulate most often on the horizontal surfaces. The copulation time in *I. persulcatus* appeared to be longer when performed on the horizontal surface whereas the time of insemination in *I. ricinus* was almost equal irrespective of the type of the surface offered. In *I. ricinus* both males and females are attracted to each other by guanine [Guerin et al., 1999] and copulate on the horizontal surfaces. This is probably the main explanation of the known phenomenon of 100% level of insemination of *I. ricinus* females collected

in the field [Babenko, 1985]. *I.persulcatus* ticks collected on certain plants demonstrate the positive reactions to some plant odors [Pomerantsev, Serdyukova, 1948]. Such reactions can also depend on the infection intensity with tick-borne encephalitis virus [Aleksseev et al., 1988, 1991, 1992]. The demonstrated attraction of both sexes to certain plant odors can be counted as an argument for the tick preference of vertical surfaces and the active search of greater heights at which the coupling can be most probable.

The infestation of one or both mating ticks with *Borrelia* increases their copulation time and decreases their speed and the height of climbing up (Figs. 3, 5, B). In *I.ricinus* individuals do not copulate when heavily infected with *B.afzelii* (Fig. 4). This fact may explain the rare sexual transmission of this species of *Borrelia* by *I.ricinus*. *I.persulcatus* ticks are able to transmit *Borrelia* sexually. They transmit *B.garinii* mainly, if not exclusively, from the male to female. The transovarial transmission of *B.garinii* was proven by finding the infected individuals (4.5%) among the unfed tick larvae collected from the vegetation. The earlier reported 100% infestation of ticks by Balashov and Grigoryeva [1998] can be an artifact. The data discussed hereabove stress one more time the importance of studying the sexual behavior of Ixodoidea ticks as a tool for understanding the pathogen circulation in the populations of different species of ticks.

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