Phenology of *Ixodes ricinus* and Infection with *Borrelia burgdorferi* sensu lato Along a North- and South-Facing Altitudinal Gradient on Chaumont Mountain, Switzerland

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ABSTRACT Questing *Ixodes ricinus* L. ticks were collected monthly from 2003 to 2005 on the north- and south-facing slopes of Chaumont Mountain in Neuchâtel, Switzerland, at altitudes varying from 620 to 1,070 m. On the south-facing slope, questing tick density was higher than on the north-facing slope, and it decreased with altitude. Density tended to increase with altitude on the north-facing slope. Saturation deficit values higher than 10 mmHg and lasting for >2 mo were often recorded on the south-facing slope, explaining seasonal patterns of questing tick activity. The overall prevalence of *Borrelia burgdorferi* sensu lato was 22.4%, and prevalence differed according to exposure and among years. No difference was noticed between nymphs and adults. Four *Borrelia* species were identified. Mixed infections were detected in 52 ticks, *B. garinii* and *B. valaisiana* ($n = 21$) and *B. afzelii* and *B. burgdorferi* s.s. ($n = 20$) were the most frequent associations observed. The density of infected ticks varied from 3.6 to 78.7 infected nymphs per 100 m^2 and from 0.6 to 16.9 infected adults per 100 m^2, both slopes combined. The study on the south-facing slope was a follow-up of a previous study carried out at the same location during 1999–2001. Comparison of climatic data between the two periods showed a marked increase in saturation deficit. Substantial differences in density and phenology of ticks also were observed. At high elevations, ticks were significantly more abundant during the current study. This can be explained by rising temperatures recorded during summer at altitude, reaching values similar to those registered in the first study beneath. At the lowest altitude, adults were significantly less abundant, probably due to long-lasting high saturation deficits that impaired nymphal survival. The density of *Borrelia*-infected ticks was higher than in the previous study.

KEY WORDS tick density, *Borrelia*-infected ticks, phenology, climate, mixed infection

The tick *Ixodes ricinus* L. is the main vector of *Borrelia burgdorferi* sensu lato, the etiological agents of Lyme borreliosis, in Europe. Seven *Borrelia* genospecies have been found associated with this tick species: *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, *B. valaisiana*, *B. lusitaniae*, *B. bissettii*, and *B. spielmanii* (Rauter and Hartung 2005, Richter et al. 2006).

Throughout the wide distribution of *I. ricinus*, its phenology (the evolution of the questing tick density over time, Jouda et al. 2004b) varies considerably from a unimodal pattern with maximum nymphal density in winter (Algeria) or in summer (Tunisia) (Yousfi-Monod and Aeschlimann 1986, Dsouli et al. 2006) to a bimodal pattern reaching its maximum density in spring (Ireland) or in autumn (Crimea) (Gray 1984, Korenberg 2000). In England (Randolph et al. 2002) and in Switzerland (Mermod et al. 1973; Perret et al. 2000; Jouda et al. 2004a, 2004b; Burri et al. 2007), both bimodal and unimodal patterns with a maximum nymphal density in spring were shown to occur. The phenology of *I. ricinus* also was reported to be variable between years (Tälleklint and Jaenson 1996, Perret et al. 2000, Randolph et al. 2002, Randolph 2004, Jouda et al. 2004b). Variations in the seasonal activity of questing ticks may be associated with variations in biotic (host species, density and behavior, and vegetation structure) as well as abiotic factors (climate). For example, the duration of development of *I. ricinus* is inversely proportional to temperature (MacLeod 1934, Campbell 1948 cited in Randolph et al. 2002); therefore, temperature may allow fast development of spring-fed ticks and the emergence of the next stage in the same year in autumn (Chmela 1969, Randolph et al. 2002). Hence, we would expect an autumnal peak when temperatures are high in summer and no autumn peak otherwise.

In a previous study on the phenology of *I. ricinus* ticks along an altitudinal gradient on the south-facing slope of a mountain (Chaumont), the seasonal activity of questing ticks was related to altitude, and tick density and prevalence of *B. burgdorferi* infection de-

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creased with altitude (Jouda et al. 2004b). In this study, variable phenologies and variable *Borrelia* infection rates of ticks were reported by Jouda et al. (2004b) at different altitudes, under different temperature conditions, within the same forest at a short distance, suggesting that such an area is suitable to follow the impact of different climatic scenarios on the seasonal activity of questing tick populations. Therefore, in the context of climate change, we followed up the seasonal activity of questing *I. ricinus* ticks as well as their infection rate with *B. burgdorferi* s.l. along this altitudinal gradient over three more years in relation with climatic data, and we investigated the situation on the north-facing slope of the same mountain.

Materials and Methods

Collection of Ticks. This study was carried out in a mixed forest dominated by deciduous trees (*Quercus* spp., *Fagus* spp., *Fraxinus* spp., and *Prunus* spp.) along two altitudinal gradients on the north- and south-facing slopes of Chaumont Mountain in Neuchâtel, Switzerland, within a 3-km radius of 47° 01′ N, 06° 56′ E. Questing ticks were collected monthly from February 2003 to November 2005 on the south-facing slope of Chaumont and from March 2004 to November 2005 on the north-facing slope. Sampling sites on the south-facing slope were located at 620, 740, and 900 m as described in Jouda et al. (2004b), and one sampling site was added at 1,070 m. Sites on the north-facing slope were chosen at similar altitudes: 780, 910, and 1,010 m. A 1-m^2 cotton flag was dragged across low vegetation over a distance of 150 m except at 620 m on the south-facing slope where flagging distance was 100 m. The flag was inspected every 25 m, and nymphs and adults were placed separately in vials containing fresh grass and kept alive (at room temperature and relative humidity close to 95%) until detection of *Borrelia* sp. infection.

Climatic Data. Environmental data such as air temperature at 60 cm and relative humidity were recorded monthly at each sampling site using a thermohygrometer (model 615, Testo SA, Lonay, Switzerland), and saturation deficit (SD), which integrates temperature and relative humidity to derive a measure of the drying power of the atmosphere, was calculated according to Randolph and Storey (1999). SD values for 1999–2001 were calculated from unpublished data recorded monthly at each sampling site during the first study. In addition, air temperature for the south-facing slope was recorded by two automatic meteorological stations (MétéoSuisse) located at 485 and 1,073 m above sea level, close to our study sites. Monthly average temperatures were considered to calculate mean values by seasons: winter (December of previous year, January and February), spring (March, April, and May), summer (June, July, and August) and autumn (September, October, and November).

Tick Phenology. Host-seeking tick density was expressed as the number of ticks collected per 100 m^2. Same variables used in the previous study at the same location (Jouda et al. 2004b) were applied to characterize tick phenology (Eisen et al. 2003, Jouda et al. 2004a). The cumulative density of ticks (CTD), expressed as the number of ticks per 100 m^2 and per year, was estimated by integrating the linearly extrapolated curve of questing tick density over 1 yr for nymphs (CTDN) and adults (CTDA). The peak tick density (PTD) is the maximal tick density over 1 yr, calculated for nymphs (PTDN) and adults (PTDA).

The onset of significant questing tick activity in spring (O10) was calculated from the questing tick density curve as the date when tick density was higher than 10% of PTD (Jouda et al. 2004b). Likewise, the end of questing tick activity (F10) was considered as the date when tick density remains constantly below 10% of PTD. To estimate maximal density of *Borrelia*-infected ticks, the peak tick density of infected nymphs (PTDNi) and adults (PTDAi) were calculated by multiplying PTD by the infection rate (Jouda et al. 2004a, 2004b).

***Borrelia* DNA Extraction from Ticks.** Every month, a maximum of 20 nymphs and 20 adults (10 males and 10 females) collected in each site were examined for *Borrelia* infection. Before DNA isolation, ticks were soaked in ethanol 70% and air-dried. Isolation of DNA from ticks collected in 2005 was achieved using ammonium hydroxide (NH_4OH) as described previously (Guy and Stanek 1991, Rijpkema et al. 1996). Briefly, ticks were boiled for 15 min at 100°C in 100 μl of 0.7 M NH_4OH, cooled quickly, and boiled again for 15 min in open vials to evaporate the ammonia (10-μl samples were retained for polymerase chain reaction [PCR] analysis). Ticks collected in 2004 were halved lengthwise: one half was processed by using ammonium hydroxide as described above (20 μl used for PCR), and the other half of the tick was incubated for 2 wk at 34°C in 1.2 ml of BSK-H culture medium (Sinsky and Piesman 1989). These cultures were washed twice with phosphate-buffered saline/$MgCl_2$, and the pellet was resuspended in 30 μl of water and heated for 15 min at 100°C according to Postic et al. (1994) (10 μl used for PCR). To check for cross-contamination, negative controls were included during DNA extraction, which consisted of reagents without ticks.

Identification of *Borrelia* Species by PCR and Reverse Line Blotting (RLB). *I. ricinus* ticks were analyzed for detection of *B. burgdorferi* s.l. genospecies by PCR and RLB. Primers B5S-Bor and 23S-Bor were used to amplify the variable spacer region between two repeated copies of the *23S* and *5S* ribosomal genes (Alekseev et al. 2001). PCR amplifications were run in a Tgradient Thermocycler 96 (Whatman Biometra, Göttingen, Germany) by using a touchdown PCR program (Burri et al. 2007). Negative and positive controls were included in each PCR. In negative controls, water replaced DNA samples. Isolates of *B. burgdorferi* ss (B31), *B. garinii* (NE11), *B. afzelii* (NE632), *B. lusitaniae* (PotiB1, PotiB2, and PotiB3), and *B. valaisiana* (VS116) were used as positive controls.

For *Borrelia* identification by RLB, PCR products were hybridized to seven oligonucleotide probes (75 pmol) (Poupon et al. 2006, Burri et al. 2007) blotted in lines on an activated Biodyne C membrane (Pall

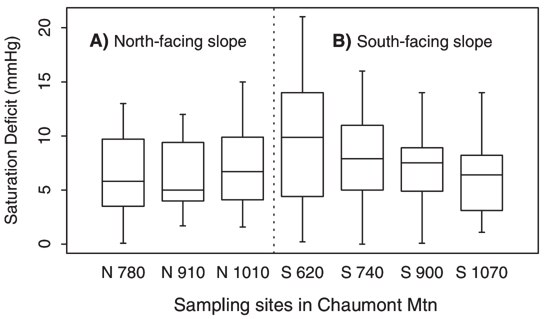

Fig. 1. Boxplot of saturation deficit values at different altitudes on both slopes of Chaumont Mountain. (A) North-facing slope (altitudes ranging from 780 to 1,010 m). Data from 2004 and 2005. (B) South-facing slope (altitudes ranging from 620 to 1,070 m). Data from 2003 to 2005.

Europe Ltd., Portsmouth, United Kingdom) by using a Miniblotter 45 (Immunetic, Cambridge, MA). Hybridization was visualized by incubating the membrane with enhanced chemiluminescence detection liquid (GE Healthcare Europe, Otelfingen, Switzerland) and by exposing the membrane to X-ray film (Hyperfilm, GE Healthcare Europe).

Statistical Analysis. The relationships between tick density and altitude, and between climatic variables (temperature, relative humidity, and SD) and altitude were evaluated with the Spearman's rank correlation. The relationship between tick density and exposure was evaluated with the unpaired Wilcoxon test. The Page test was applied to assess the relation between the onset of tick activity in spring and altitude. The chi-square goodness-of-fit test with uniform distribution was used to compare the CTD values between the earlier and the current study on the southern slope of Chaumont Mountain. The chi-square test on contingency table was used for *Borrelia* infection comparisons between stages and altitudes. The influence of exposure, altitude, and year on infection prevalence in nymphal and adult ticks was estimated by logistic regressions (only years 2004 and 2005 were taking into account). These variables were considered as factors. All statistics were calculated with S-Plus 7.0 for Windows (Insightful, Reinach, Switzerland).

Results

Climatic Data. Analysis of climatic data recorded at Chaumont Mountain showed a different pattern on both slopes of the mountain. On the south-facing slope, relative humidity increased with altitude, whereas SDs were inversely related to altitude (Spearman's rank correlation, $P = 0.046$ for relative humidity

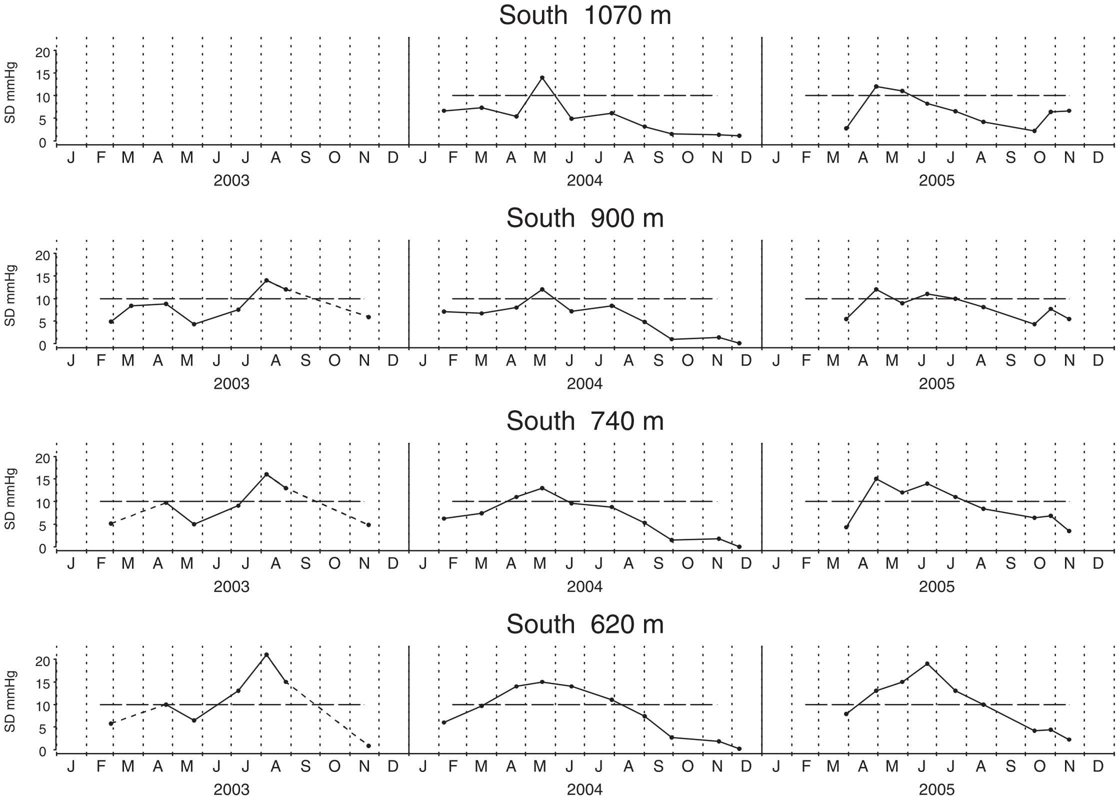

Fig. 2. Seasonal evolution of saturation deficit (SD, in mmHg) along an altitudinal gradient on the south-facing slope of Chaumont Mountain. from 2003 to 2005. The broken lines indicate the threshold of 10 mmHg.

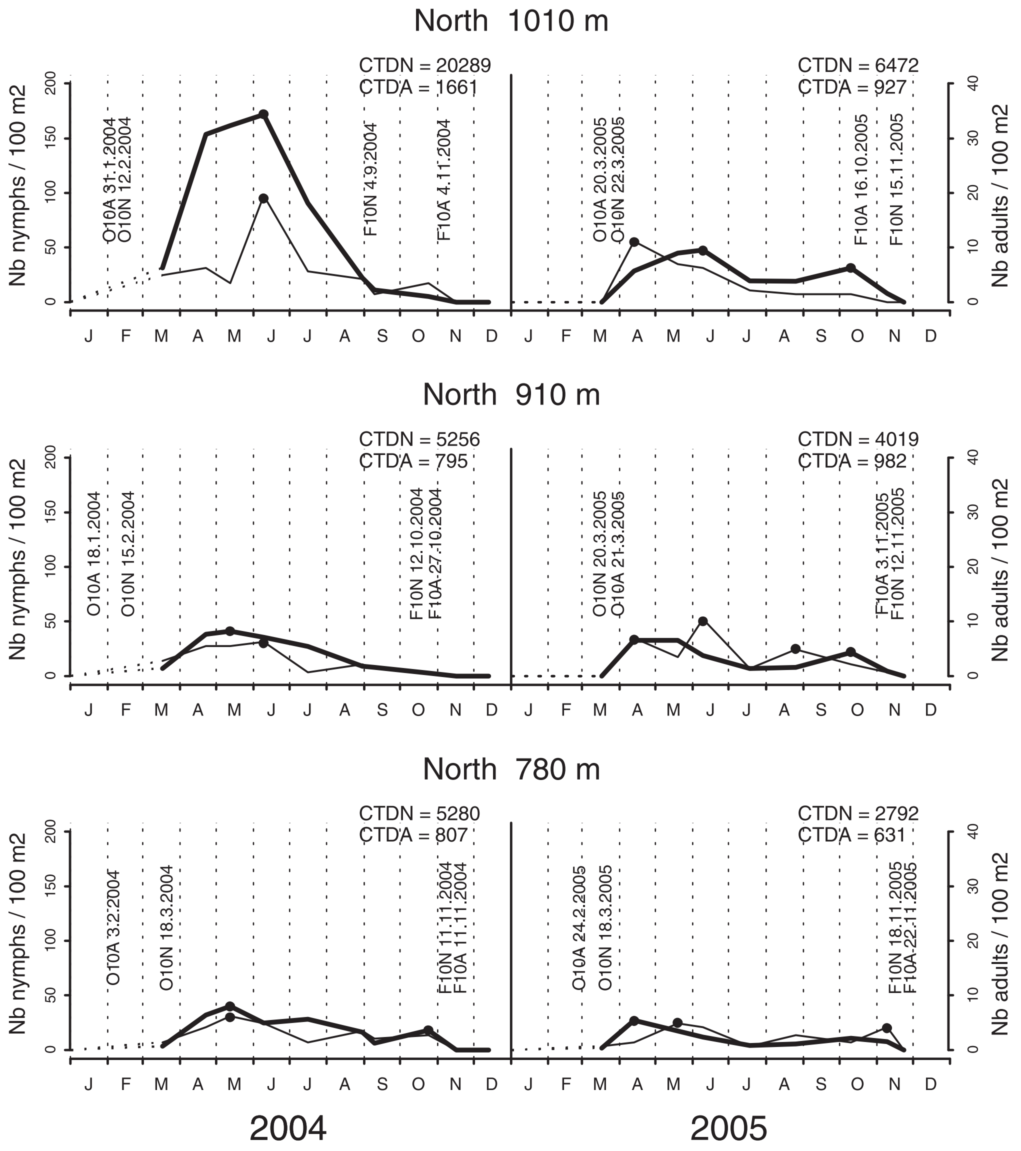

Fig. 3. Questing *I. ricinus* nymphs (bold line) and adults (thin line) at three different altitudes on the north-facing slope of Chaumont Mountain from 2004 to 2005. CTDN, number of nymphs per 100 m^2 per year. CTDA, number of adults per 100 m^2 per year. O10N, onset of nymphal activity. O10A, onset of adult activity. F10N, end of nymphal activity. F10A, end of adult activity.

and $P = 0.023$ for SD). Temperature tended to decrease with altitude (Spearman's rank correlation, $P = 0.07$). On the north-facing slope, no correlations were observed between climatic variables and altitude. On the south-facing slope, SD median values were between 6 and 10 mmHg, whereas these values were usually <6 mmHg on the north-facing slope (Fig. 1). Even if we excluded data from the hottest year (2003) on the south-facing slope, SD median values were always higher than 6 mmHg (values varied between 6.14 and 9.8 mmHg).

On the south-facing slope, at 620 and 740 m, SD values >10 mmHg and lasting for 2–4 mo from June to September were observed in 2003, whereas in 2004 and 2005 these high SD values were already present from March-April and lasted for 4 mo at 620 m (Fig. 2). The maximum SD value was observed at 620 m on the south-facing slope reaching 21.6 mmHg in 2003. At the two highest altitudes, SD values rose above 10 mmHg only for short periods, except at 900 m in 2003 where SD remained higher than 10 mmHg for 2 mo. On the north-facing altitudinal gradient, SD values rarely exceeded 10 mmHg (data not shown).

Phenology, Tick Density, and *Borrelia* Infection on South- and North-Facing Slopes. The majority of questing ticks was collected at temperatures >8°C for

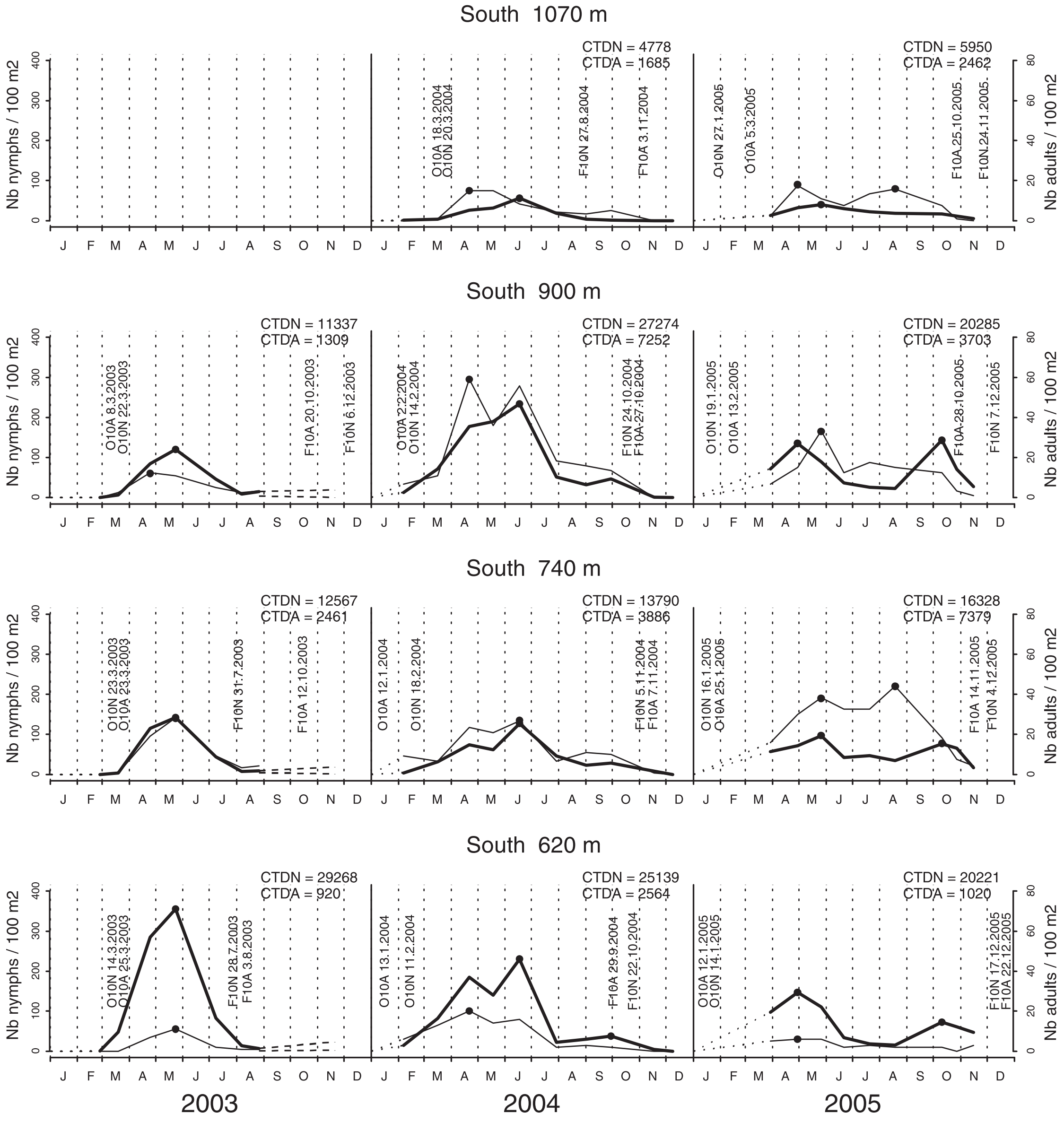

Fig. 4. Questing *I. ricinus* nymphs (bold line) and adults (thin line) at four different altitudes on the south-facing slope of Chaumont Mountain from 2003 to 2005. CTDN, number of nymphs per 100 m^2 per year. CTDA, number of adults per 100 m^2 per year. O10N, onset of nymphal activity. O10A, onset of adult activity. F10N, end of nymphal activity. F10A, end of adult activity.

nymphs and 8.5°C for adults (data not shown). However, nymphs could occasionally be collected already at 2.9°C and adults at 4.2°C.

On both slopes of Chaumont, the seasonal patterns of questing tick activity varied according to year and to altitude (Figs. 3 and 4). On the north-facing slope, larvae peaked in June in all sites. On the south-facing slope, questing activity of larvae and nymphs reached their maximum simultaneously in May to July according to altitudes, except at the highest altitude where the larval peak occurred 1 mo after the nymphal peak (data not shown for larvae). On the north-facing slope, seasonal nymphal and adult questing activity patterns were usually stable, except in 2004 at 1,010 m with the emergence of a strong activity peak in spring (Fig. 3). On the south-facing slope, seasonal patterns were stable at 1,070 m, whereas they were changeable among years at the other elevations (Fig. 4).

In 2003, unimodal tick activity patterns were observed along the south-facing altitudinal gradient (no data for the north-facing slope) (Fig. 4). In 2005, a clear bimodal distribution of questing tick activity was noticed in almost all study sites on both slopes of the mountain with peaks of similar magnitude in spring

Table 1. Proportion of *B. burgdorferi* sl infected *I. ricinus* ticks at different altitudes on the north- and south-facing slopes of Chaumont Mountain from 2003 to 2005

Site/altitude (m)	Infection rates in nymphs (%)				Infection rates in adults (%)			
	2003	2004	2005	Total	2003	2004	2005	Total
N 1,010	na	37/140 (26.4)	32/116 (27.6)	69/256 (26.9)	na	9/59 (15.3)	3/40 (7.5)	12/99 (12.1)
N 910	na	12/112 (10.7)	11/101 (10.9)	23/213 (10.8)	na	5/34 (14.7)	11/39 (28.2)	16/73 (21.9)
N 780	na	23/131 (17.6)	21/90 (23.3)	44/221 (19.9)	na	8/38 (21.1)	4/28 (14.3)	12/66 (18.2)
Total N	na	72/383 (18.8)	64/307 (20.8)	136/690 (19.7)	na	22/131 (16.8)	18/107 (16.8)	40/238 (16.8)
S 1,070	0/5 (0.0)	31/88 (35.2)	25/123 (20.3)	56/216 (25.9)	0/2 (0.0)	12/49 (24.5)	17/69 (24.6)	29/120 (24.2)
S 900	14/86 (16.3)	40/140 (28.6)	23/149 (15.4)	77/375 (20.5)	7/30 (23.3)	19/107 (17.8)	25/94 (26.6)	51/231 (22.1)
S 740	16/75 (21.3)	46/144 (31.9)	16/164 (9.8)	78/383 (20.4)	11/43 (25.6)	38/91 (41.8)	25/137 (18.2)	74/271 (27.3)
S 620	22/100 (22.0)	54/158 (34.2)	36/154 (23.4)	112/412 (27.2)	0/5 (0.0)	21/59 (35.6)	3/22 (13.6)	24/86 (27.9)
Total S	52/266 (19.5)	171/530 (32.3)	100/590 (16.9)	323/1386 (23.3)	18/80 (22.5)	90/306 (29.4)	70/322 (21.7)	178/708 (25.1)
Total N & S	52/266 (19.5)	243/913 (26.6)	164/897 (18.3)	459/2076 (22.1)	18/80 (22.5)	112/437 (25.6)	88/429 (20.5)	218/946 (23.0)

N, north-facing slope; S, south-facing slope; na, not available.

and autumn (Figs. 3 and 4). Tick density in autumn was sometimes even higher than in spring as seen for adults at 740 m and for nymphs at 900 m on the south-facing slope.

Altogether, cumulative tick density was significantly higher on the south-facing slope than on the north-facing slope (Figs. 3 and 4) (unpaired Wilcoxon test, $n = 17$, $P = 0.036$). Density for nymphs decreased with altitude on the south-facing slope (Spearman's rank correlation, $P = 0.048$), but it had a tendency to increase with altitude on the north-facing slope of Chaumont (Spearman's rank correlation, $P = 0.09$). The beginning of nymphal activity in spring (ON10) was delayed with altitude on the south-facing slope (Page test, $P < 0.05$) but not on the northern slope.

In total, 459/2,076 (22.1%) nymphs and 218/946 (23.0%) adults analyzed on both altitudinal gradients were infected by *B. burgdorferi* s.l. (Table 1). There were no significant differences in the infection prevalences between nymphal and adult stages either between the seven examined sites (χ^2 test, $P = 0.6$) or when the north- and south-facing slopes were considered (χ^2 test, $P = 0.37$ and $P = 0.38$, respectively).

Borrelia prevalences in ticks varied according to exposure (higher on the south-facing slope of Chaumont) and among years on the south-facing slope (maximal prevalence in 2004) (logistic regression, nymphs: $t = 2.257$, $P = 0.024$; adults: $t = 2.592$, $P = 0.009$ for exposure; logistic regression, nymphs: $t = -5.789$, $P < 0.0001$; adults: $t = -2.032$, $P = 0.042$ for year). Infection prevalences in nymphs varied according to altitude, higher at the lowest altitude on the south-facing slope (620 m) (logistic regression, $t = -2.275$, $P = 0.023$) and higher at the highest altitude (1,010 m) on the north-facing slope (logistic regression, $t = 3.823$, $P = 0.0001$). During the current study, the PTDi varied from 3.6 to 78.7 infected nymphs per 100 m^2 (Table 2) and was five-fold lower for adults, ranging between 0.6 and 16.9 infected adults per 100 m^2.

Four *Borrelia* species were identified by RLB in ticks analyzed in 2004 ($n = 1,350$) and 2005 ($n = 1,326$): *B. afzelii*, *B. garinii*, *B. burgdorferi* s.s., and *B. valaisiana*. *B. afzelii* was usually the dominant species at each site with the highest prevalence at 1,010 m on the north-facing slope and the lowest at 1,070 m on the south-facing slope (Table 3). At this site *B. burgdorferi* ss was the dominant species closely followed by *B. valaisiana.* On the north-facing slope *B. burgdorferi* s.s. was more frequent at 910 m and *B. garini* and *B. valaisiana* at 780 m. Mixed infections were detected in 52 ticks (29 nymphs and 23 adults): *B. garinii* and *B. valaisiana* in 21 ticks, *B. afzelii* and *B. burgdorferi* s.s. in 20 ticks, *B. garinii* and *B. afzelii* in six ticks, *B. garinii* and *B. burgdorferi* s.s. in three ticks, and *B. afzelii* and

Table 2. Peak tick density (PTD)[a] and peak tick density of infected *I. ricinus* ticks (PTDI)[b] at different altitudes on the north- and south-facing slopes of Chaumont Mountain from 2003 to 2005

Site/altitude (m)	Nymphs						Adults					
	PTD			PTDI			PTD			PTDI		
	2003	2004	2005	2003	2004	2005	2003	2004	2005	2003	2004	2005
N 1,010	na	172	47	na	45.4	13.0	na	19	11	na	5.0	3.0
N 910	na	41	33	na	4.4	3.6	na	6	10	na	0.6	1.1
N 780	na	40	27	na	7.0	6.3	na	6	5	na	1.1	1.2
S 1,070	na	56	40	na	19.7	8.1	na	15	18	na	5.3	3.7
S 900	120	234	143	19.6	66.9	22.0	12	59	33	2.0	16.9	5.1
S 740	142	127	97	30.2	40.5	9.5	28	27	44	6.0	8.6	4.3
S 620	355	230	147	78.1	78.7	34.4	11	20	6	2.4	6.7	1.4

N, north-facing slope; na, not available; S, south-facing slope.

[a] PTD, maximal tick density over 1 yr expressed as number of ticks per 100 m^2.

[b] PTDi: PTD x infection prevalence. Maximal infected tick density over 1 yr expressed as number of infected ticks per 100 m^2.

Table 3. Distribution and prevalence of *B. burgdorferi* sl species in infected *I. ricinus* ticks on the north- and south-facing slopes of Chaumont Mountain in 2004 and 2005

Site/altitude (m)	Nymphs							Adults						
	Borrelia Identification (%)[a]						Infected/tested (%)	*Borrelia* Identification (%)						Infected/tested (%)
	af	ga	ss	vs	sl	mixed		af	ga	ss	vs	sl	mixed	
N 1,010	50	5	3	3	4	4	69/256 (27.0)	4	2	3	2	1	0	12/99 (12.1)
N 910	12	2	3	2	3	1	23/213 (10.8)	5	2	5	1	1	2	16/73 (21.9)
N 780	13	10	2	7	8	4	44/221 (19.9)	3	1	1	3	2	2	12/66 (18.2)
Total N	75 (55.2)	17 (12.5)	8 (5.9)	12 (8.8)	15 (11.0)	9 (6.6)	136/690 (19.7)	12 (30.0)	5 (12.5)	9 (22.5)	6 (15.0)	4 (10.0)	4 (10.0)	40/238 (16.8)
S 1,070	12	10	13	11	6	4	56/211 (26.5)	4	4	8	6	4	3	29/118 (24.6)
S 900	34	6	10	5	4	4	63/289 (21.8)	16	7	10	3	3	5	44/201 (21.9)
S 740	30	11	6	2	6	7	62/308 (20.1)	14	10	13	9	9	8	63/228 (27.6)
S 620	53	15	4	4	9	5	90/312 (28.8)	10	6	0	1	4	3	24/81 (29.6)
Total S	129 (47.6)	42 (15.5)	33 (12.2)	22 (8.1)	25 (9.2)	20 (7.4)	271/1120 (24.2)	44 (27.5)	27 (16.9)	31 (19.3)	19 (11.9)	20 (12.5)	19 (11.9)	160/628 (25.5)
Total N & S	204 (50.1)	59 (14.5)	41 (10.1)	34 (8.4)	40 (9.8)	29 (7.1)	407/1810 (22.5)	56 (28.0)	32 (16.0)	40 (20.0)	25 (12.5)	24 (12.0)	23 (11.5)	200/866 (23.1)

N, north-facing slope, S, south-facing slope.

[a] af, *B. afzelii;* ga, *B. garinii;* ss, *B. burgdorferi* s.s.; vs, *B. valaisiana;* sl, untypeable *Borrelia;* mixed, mixed *B. burgdorferi* s.l. infection.

B. valaisiana, and *B. burgdorferi* ss, *B. garinii,* and *B. afzelii* in one tick each.

Evolution of Tick Densities and Seasonal Activity on the South-Facing Slope between Two Study Periods. Comparison of climatic data on the south-facing slope between the previous study carried out in 1999–2001 (Jouda et al. 2004b) and the current study showed a marked increase in SD in 2003–2005. During the first study, the threshold of 10 mmHg was rarely reached even at the lowest altitudes and only for very short periods. This threshold happened punctually after the spring peak (in September 1999 and in July 2000) or earlier (end of May 2001 until mid-July). Between the two studies, differences were observed in monthly average temperature as recorded by the MétéoSuisse stations (Table 4). Monthly average temperatures during winter and spring decreased between the two periods, whereas monthly average temperature in summer and autumn increased. This evolution belongs to a general trend observed during 1996–2005. The major temperature increase was recorded at the highest altitude: mean summery temperatures were 1.6°C above the 1999–2001 values. A closer look at the monthly average temperatures showed that the main decreases occurred in February (2.31°C at 485 m and 1.97°C at 1,073 m) and in May (1.83°C at 485 m and 1.73°C at 1,073 m), and the main increases took place in June (2.90°C at 485 m and 3.13°C at 1,073 m) and July (1.13°C for both stations).

Substantial variations in density and phenology of ticks were observed between the two studies. At high elevations (740 and 900 m), CTD values for nymphs and adults were significantly higher during the last period (chi-square goodness-of-fit test, $P < 0.0001$) than during the first period (Table 5). The most important divergence was observed at 900 m where mean CTD values were 2.25 and 3.5 times higher for nymphs and adults, respectively, during the current study. At the lowest altitude, no difference was observed for nymphs, whereas adults were significantly less abundant during the last period (chi-square goodness-of-fit test, $P < 0.0001$). In addition, the seasonal activity of ticks at 900 m displayed a greater variability between years compared with the first study.

Discussion

A previous study along an altitudinal gradient on the south-facing slope of Chaumont revealed that phenology was related to altitude and that tick density and prevalence of *B. burgdorferi* infection decreased with altitude (Jouda et al. 2004b). Different temperature conditions occurring simultaneously within a short distance along this altitudinal gradient induced variable phenologies of ticks. Hence, modifications of temperature in the context of global warming may help to better understand their influence on the evolution of questing tick density and Lyme borreliosis risk. Therefore, we followed up this study over three more years considering climatic factors, and we investigated the situation

Table 4. Seasonal average temperatures (°C) during the two study periods (1999–2001, Jouda et al. [2004b] and 2003–2005) and during the 3-yr period preceding the first study recorded at two meteorological stations located at 485 and 1,073 m on the south-facing slope of Chaumont Mountain

Season	Station at 485 m				Station at 1,073 m			
	1996–1998	1999–2001	2003–2005	Difference[a]	1996–1998	1999–2001	2003–2005	Difference*
Spring	9.9	10.6	10.1	−0.5	5.6	6.2	5.9	−0.3
Summer	18.4	18.5	20.0	1.5	13.6	13.9	15.5	1.6
Autumn	9.6	10.5	12.0	1.5	5.8	6.6	7.1	0.5
Winter	2.0	2.7	1.6	−1.1	−0.4	−0.7	−1.7	−1.0
Annual avg	10.0	10.6	10.9	0.3	6.2	6.5	6.7	0.2

[a] Differences were calculated between 1999–2001 and 2003–2005.

along an altitudinal gradient on the north-facing slope of the same mountain.

An annual variability in tick density (CTD) as well as changeable patterns of tick activity between years was noticed in all study sites. This is in line with previous temporal studies on tick density reported in Europe (Tälleklint and Jaenson 1996, Jensen 2000, Jensen and Frandsen 2000, Randolph et al. 2002, Randolph 2004, Jouda et al. 2004b, Ferquel et al. 2006).

On the south-facing slope of the mountain, a unimodal distribution of tick activity was observed in 2003. Interestingly, this summer extreme heat and low precipitations led to an exceptionally dry season over most parts of Europe, particularly in western Switzerland (Rebetez et al. 2006). SD values above 10 mmHg were recorded after June and remained high for months. High SD causes water stress in ticks (especially in nymphs) so questing activity is generally interrupted and ticks move to the ground for rehydration. Moreover, rising tick movements due to high SD may increase tick mortality owing to exhaustion of their energy reserves (Randolph and Storey 1999; Perret et al. 2003, 2004). Thus, this exceptional increase in summer temperatures in 2003 and the high and long-lasting SD values may have impaired questing tick activity and increased tick mortality, preventing the occurrence of an autumn peak at short term and reducing tick density at long term.

The second remarkable event was the appearance of a low spring peak of questing tick activity and a marked autumn peak in 2005 at the three lowest elevations on the south-facing slope. Probably, the weak spring peak of questing tick may have been induced by high SD values exceeding 10 mmHg early in spring and remaining high until August. We do not think that the low winter temperatures recorded the same year are responsible for this reduced questing tick density in spring. Moreover, snow cover prevents low soil temperatures (Berry 1981 cited in Lindgren et al. 2000) and protects ticks from very cold temperatures and probably does not have severe consequences for ticks, which are quiescent at this period (Randolph 2004). The autumn peaks, which are as important as spring peaks, are probably due to two phenomena. Favored development of spring-fed larvae and nymphs at all altitudes due to an increase in summer temperatures allowed these ticks to quest in autumn the same year. In addition, spring ticks may have reactivated their questing activity in autumn. Interestingly, the autumn peak emerged when SD values dropped. A similar phenomenon was described in 1998 in a very close site (Perret et al. 2000).

Table 5. Cumulative tick density (CTD) of *I. ricinus* ticks calculated for the 1999–2001 and 2003–2005 surveys on the south-facing slope of Chaumont Mountain

Altitude (m)	CTD Nymphs		CTD Adults	
	1999–2001[a]	2003–2005	1999–2001[a]	2003–2005
900	26,177	58,896	3,477	12,264
740	28,923	42,685	6,830	13,726
620	74,990	74,628	7,789	4,504

[a] 1999–2001: Data from Jouda et al. (2004b).

Recorded climatic data did not allow explaining changeable patterns of tick activity in sites with northern exposure, especially at 1,010 m in 2004 when a noteworthy spring peak of questing ticks occurred. Moreover, the effect of increased temperature, as recorded in summer 2003, on the behavior of some host communities that might influence tick densities is unknown.

Questing tick density decreased with altitude on the south-facing slope of Chaumont, which is in line with previous observations (Jouda et al. 2004b). On the north-facing slope, the highest tick density was recorded at the highest altitude, >1,000 m. This situation contrasts with a study describing that tick establishment and development were limited or prevented by cold temperatures at high altitudes (Daniel 1993). However, higher tick density in altitude also has been observed along a north-facing altitudinal gradient but in an area with a dry and warm climate, the Rhône Valley in Switzerland (Burri et al. 2007). Local differences in micro- and mesoclimatic conditions may allow a better maintenance at high altitudes than at low altitudes.

When we compared our results from the south-facing slope with those from the previous study (1999–2001), striking differences were observed in tick phenology and this during a period of climate features showing warmer summers and autumns and colder winters. Considering a longer period (1996–2005), annual average temperatures showed an increase of 0.9 and 0.5°C at the lowest and highest altitudes, respectively, and this within a clear trend of warmer summers and autumns and colder winters. The temperatures recorded at 485 m from 2003 to 2005 (10.9°C) were higher than the average annual temperature calculated over the 20th century, which was 9.4°C (Baniewicz 2002). Evolution of seasonal questing tick activity and tick densities at 900 m on the south-facing slope demonstrated great variability, whereas stability was the trend registered in the previous study at this altitude. This instability in tick phenology under warmer conditions at high altitude was predicted by Jouda et al. 2004b). This can be explained by rising temperatures recorded in summer at altitude, reaching values similar to those registered in the first study at the lowest altitude and that are favorable for ticks, increasing their density at 900 m. Similar effects of warm temperature on tick population at high elevation have been described by Daniel et al. (2003) and Danielova et al. (2006). They showed an extension of *I. ricinus* distribution at higher altitudes during the last decades in the Czech Republic, probably due to an increase in temperatures.

Otherwise, at the lowest altitude, high temperatures and moisture stress had a considerable negative impact on tick abundance. Long-lasting high SD values as observed during the last study probably represent a danger for tick population survival. At this altitude, ticks seem to suffer from the effect of global warming, and possibly, they are reaching here the limit of the climatic conditions tolerable for their maintenance. A similar phenomenon also was noticed in the Swiss Alps, in a warmer and drier region (Burri et al. 2007) where tick establishment was prevented by high and long-lasting SD values. Nymphs show lower resistance to water stress than adults, leading to increased death rates (Perret et al. 2004). Probably, nymphs could not quest long enough to find a host before dying of desiccation and/or energy exhaustion. This could explain the lower adult densities recorded during the current study.

Upon synchrony of extreme weather conditions with the tick questing activity, for example, in spring at the lowest altitude, whereas high and long-lasting SDs arose and many ticks quested, questing duration was reduced and tick mortality was increased, leading to a lower questing tick population (Perret et al. 2003, 2004). At higher altitudes, increased temperatures to values favorable to ticks led to an increase in tick density probably through reduced developmental duration from one instar to the next. In both scenarios, it remains to elucidate whether the host community was affected by temperature changes and how this might influence questing tick population.

The overall prevalence of *B. burgdorferi* s.l. in questing *I. ricinus* ticks was 22.4% (677/3,022) and differed considerably among years. Our data are in line with those previously reported in Switzerland (Péter et al. 1995; Jouda et al. 2003, 2004a; Burri et al. 2007) and more particularly in Neuchâtel (Aeschlimann et al. 1986, Jouda et al. 2004b). Curiously, no significant difference was detected between nymphal and adult infection rates as reported previously in this site (Jouda et al. 2004b). We noticed that nymphal population suffered from unfavorable climatic conditions at the lowest altitude, resulting in a smaller adult population. Whether *Borrelia* infection has an influence on tick survival under such conditions is unknown.

Infection prevalence was lowest at the highest altitude on the south-facing slope as observed in other studies (Rizzoli et al. 2002, Jouda et al. 2004b, Burri et al. 2007). However, on the north-facing slope, highest infection rates occurred at the highest altitude, reasons for such a discrepancy are unclear.

Density of infected nymphs (calculated as PTDi) was five-fold higher than density of infected adults (ranging from 3.6 to 78.7 infected nymphs per 100 m^2 versus 0.6–16.9 infected adults per 100 m^2). Maximal values surpassed those previously reported on the south-facing slope of the mountain (68.6 for nymphs and 16.7 for adults, Jouda et al. 2004b) and are among the highest notified in Switzerland (Jouda et al. 2004a, Burri et al. 2007). In France, PTDi for nymphs (calculated from data in Ferquel et al. 2006) reached higher values (106.9 infected nymphs), whereas in Sweden, PTDi was lower (6.5 infected nymphs/100 m^2, calculated from data in Tälleklint and Jaenson 1996). Usually, nymphs represent the stage of highest risk in Lyme borreliosis incidence. In Chaumont, a high nymph density and a high infection rate, both increasing, suggest an important risk for humans.

Molecular analysis of bloodmeal sources in ticks collected on both slopes of Chaumont Mountain showed that artiodactyls, red squirrels, birds, small rodents, and foxes were the main hosts for ticks (F.M.C. et al., unpublished data). This study also reported an association between host DNA and some *Borrelia* species in ticks. On both slopes, *B. afzelii* was associated with detection of rodent DNA in ticks and *B. garinii* and *B. valaisiana* with bird DNA. These hosts are known to be reservoirs for these genospecies (Kurtenbach et al. 1998; Humair and Gern 1998; Humair et al. 1998, 1999).

The great variability of infection prevalence (higher on the south-facing slope) and distinct distribution of *Borrelia* genospecies (related to exposure and altitude) at mesoclimatic scale underlines the large diversity of factors involved in *Borrelia* species prevalence in nature. *Borrelia* cycles are maintained in nature by a wide range of hosts that contribute differentially according to their competence for pathogen transmission (Piesman and Gern 2004). Understanding the evolution of tick population and tick-borne disease dynamics is essential to establishing human risk. For example, it has been predicted under climatic changes that enzootic cycles of tick-borne encephalitis virus in Europe may disappear from the southern edge of its distribution, whereas new foci will move into higher latitudes (Randolph 2001). Here, we observed an increase in the density of *Borrelia*-infected nymphs particularly at the highest altitudes over a period of 7 yr in a context of warmer summers and autumns. Global warming has an impact in this model, but it is still unclear why divergences are present depending on geographic areas and even at local scale. This is our incentive to continue to survey tick population on the south-facing slope of Chaumont in the context of global warming.

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