Comparison of the cold hardiness of *Ixodes nipponensis* and *I. persulcatus* (Acari: Ixodidae) in relation to the distribution patterns of both species in the Chichibu Mountains

Kazuyoshi FUJIMOTO*

Department of Medical Zoology, Saitama Medical School,
Moroyama-machi, Saitama 350-04, Japan

(Received: 23 June 1994; Accepted: 2 September 1994)

Key words: *Ixodes nipponensis*, *Ixodes persulcatus*, cold hardiness, Chichibu Mountains, distribution.

Abstract: In order to compare the cold hardiness of the two ticks, *I. nipponensis* and *I. persulcatus*, the survival of each stage of both species was observed at 25°C after exposure to low temperatures (4, -2, and -5°C) for 10-50 days. Both in *I. nipponensis* and in *I. persulcatus*, the egg hatchability was low (0-62.3%) when the eggs were exposed to 4 or -2°C for 10-50 days. None of the unfed larvae of either species died with exposure to -2°C for 10 days. After exposure to -2°C for 30-50 days or -5°C for 10-50 days, however, the survival rate of the unfed larvae became lower in *I. nipponensis* than in *I. persulcatus*. The molting percentage of engorged larvae of both species was high (76.7-93.3%) when they were exposed to 4°C for 10-50 days. After exposure to -2°C for 20-50 days or -5°C for 20-30 days, however, the molting percentage of engorged *I. nipponensis* larvae became lower than that of engorged *I. persulcatus* ones. The survival rate of unfed nymphs of both species was high (72.1-100%) even after exposure to -5°C for 10-50 days. The engorged nymphs of both species successfully molted (61.9-100%) with exposure to 4 or -2°C for 10-50 days. After exposure to -5°C for 10-30 days, however, engorged *I. nipponensis* nymphs were more seriously impaired than the engorged *I. persulcatus* nymphs. The high tolerance of *I. persulcatus* to low temperatures explains why this tick can survive in the upper montane to subalpine zones of the Chichibu Mountains. However, the reasons why the distribution of *I. nipponensis* is limited in the lower montane zone can not be fully explained by the present results because of the high tolerance of the unfed nymphs to low temperatures.

INTRODUCTION

The two ticks, *Ixodes nipponensis* Kitaoka and Saito and *I. persulcatus* Schulze, are

known to be closely related to each other (Kitaoka and Saito, 1967). In the Chichibu Mountains, however, the distribution of these two species was vertically separated; *I. nipponensis* was found in the lower montane zone (about 50-500 m above sea level),

* 藤本和義: 埼玉医科大学医動物学教室 (〒350-04 埼玉県入間郡毛呂山町毛呂本郷 38)

whereas *I. persulcatus* occurred in the upper montane to subalpine zones (about 600–1,800 m above sea level) (Fujimoto and Yamaguti, 1990). Takada (1990) also reported that *I. persulcatus* was found at the mountain zone in the southern part of Japan, while *I. nipponensis* occurred at the basal zone. In general, the migration and dispersal ability in ticks is very low because they are not able to fly. Therefore, the temperature in the habitat where ticks dropped from hosts has considerable effect on their development and survival. Fujimoto (1992) found that *I. persulcatus* was less tolerant to high temperatures than *I. nipponensis* in the previous study on the effects of temperature on the oviposition and development. However, this report made no mention of the tolerance of the two ticks to low temperatures. In Saitama Prefecture, the difference in winter temperature between the low plains and mountainous regions is great. For example, the mean temperatures in January (the coldest month) at Hanno (about 100 m above sea level), Chichibu (about 200 m), Nakatsugawa (about 800 m), and Mitsumine (about 1,100 m) are 3.1, 1.5, -1.2, and -2.7°C, respectively, according to the data from the Kumagaya local meteorological observatory. In this study, the cold hardiness of *I. nipponensis* and *I. persulcatus* was compared in order to clarify the role of low temperatures on the distribution patterns of the two ticks in the Chichibu Mountains.

MATERIALS AND METHODS

Origin and rearing procedures. *I. nipponensis* ticks derived from engorged nymphs detached from lizards of the species *Takydromus tachydromoides* (Schlegel) which were collected in a hilly region of Saitama Prefecture. The adult offspring in the laboratory (25°C, 16L:8D photoperiod) were used in this study. *I. persulcatus* ticks derived from adults collected by the flagging method in Yamanashi Prefecture. Both in *I. nipponensis* and in *I. persulcatus*, adults were fed on the ears of domestic rabbits, and larvae and nymphs on albino mice in the laboratory.

The engorged ticks detached were placed in tick containers (Petri dishes 3–4.5 cm in diameter) with wet filter papers at the bottom, and kept in an incubator (25°C, 16L:8D photoperiod).

Exposure of eggs, engorged larvae and nymphs to low temperature. The engorged ticks detached from the host on the same day were placed together in batches of 10 to 20 individuals in tick containers for larvae, and one to 7 individuals for nymphs, and kept in dark refrigerators at three constant temperatures (4, -2, and -5°C). Eggs were collected daily from ovipositing females kept in an incubator (25°C, 16L:8D photoperiod). The eggs collected were transferred to tick containers and kept in dark refrigerators. Eggs, engorged larvae and nymphs were placed for 10–50 days in dark refrigerators and subsequently transferred to an incubator (25°C, 16L:8D photoperiod). The percentages of normally hatched and molted individuals were calculated. Eggs, engorged larvae and nymphs were considered dead if they did not molt or hatch within a month.

Exposure of unfed larvae and nymphs to low temperature. Unfed larvae and nymphs (2- to 7-week-old ticks) placed respectively in batches of 10 to 20 individuals in tick containers were kept in dark refrigerators (-2 and -5°C) for 10–50 days and subsequently transferred to the incubator (25°C, 16L:8D photoperiod). The numbers of living larvae and nymphs were counted to determine the survival rate, respectively. Unfed larvae and nymphs were considered dead if they did not move when the worker breathed on them.

RESULTS

Tolerance of eggs to low temperatures

As shown in Table 1, the hatchability of *I. nipponensis* eggs was relatively high (46.7%) when the eggs were exposed to 4°C for 10 days. At 4°C for 30–50 days or -2°C for 10–20 days, however, only 1.0–11.8% of the eggs hatched, and none hatched at -2°C for 30–50 days.

I. persulcatus eggs were also sensitive to

Table 1 Hatchability of eggs of *I. nipponensis* and *I. persulcatus* reared at 25°C and ca. 100% RH after the low temperature treatments at ca. 100% RH.

Duration of treatments	<i>I. nipponensis</i>		<i>I. persulcatus</i>	
	No. of eggs examined	Hatchability (%)	No. of eggs examined	Hatchability (%)
Control (25°C)	929	77.2	451	97.3
4°C				
For 10 days	321	46.7	346	31.8
For 20 days	364	29.4	556	25.2
For 30 days	318	5.0	308	29.2
For 40 days	247	1.0	355	14.1
For 50 days	277	2.5	534	1.0
−2°C				
For 10 days	583	11.8	395	62.3
For 20 days	542	7.6	297	55.2
For 30 days	128	0	421	3.6
For 40 days	339	0	409	1.0
For 50 days	454	0	212	0

Table 2 Survival of unfed larvae of *I. nipponensis* and *I. persulcatus* reared at 25°C and ca. 100% RH after the low temperature treatments at ca. 100% RH.

Duration of treatments	<i>I. nipponensis</i>		<i>I. persulcatus</i>	
	No. of unfed larvae examined	Survived (%)	No. of unfed larvae examined	Survived (%)
−2°C				
For 10 days	41	100	35	100
For 30 days	38	26.3	37	83.8
For 50 days	38	0	34	55.9
−5°C				
For 10 days	43	58.1	40	100
For 20 days	41	43.9	40	90.0
For 30 days	81	12.3	40	85.0
For 40 days	39	2.6	41	51.2
For 50 days	41	0	40	37.5

low temperatures but less sensitive than *I. nipponensis* (Table 1). The hatchability of the eggs was relatively high (25.2–62.3%) when they were exposed to 4°C for 10–30 days or −2°C for 10–20 days, but decreased to 0–14.1% after exposure to 4°C for 40–50 days or −2°C for 30–50 days.

Tolerance of larvae to low temperatures

1) *Unfed larvae.* None of the unfed *I. nipponensis* larvae died when they were exposed to −2°C for 10 days (Table 2). However, the survival of the unfed larvae was reduced to 26.3% at −2°C for 30 days, and all unfed larvae died at −2°C for 50 days. A similar result was observed in unfed

Table 3 Molting success of engorged larvae of *I. nipponensis* and *I. persulcatus* reared at 25°C and ca. 100% RH after the low temperature treatments at ca. 100% RH.

Duration of treatments	<i>I. nipponensis</i>		<i>I. persulcatus</i>	
	No. of engorged larvae examined	% molting	No. of engorged larvae examined	% molting
Control (25°C)	272	90.1	122	96.7
4°C				
For 10 days	30	76.7	34	85.3
For 20 days	28	78.7	30	86.7
For 30 days	39	79.5	30	93.3
For 40 days	33	84.8	51	88.2
For 50 days	30	76.7	30	90.0
-2°C				
For 10 days	40	90.0	32	93.8
For 20 days	40	52.5	40	75.0
For 30 days	35	45.7	39	71.8
For 40 days	40	47.5	40	82.5
For 50 days	39	38.5	42	64.3
-5°C				
For 10 days	40	47.5	50	42.0
For 20 days	36	5.6	50	60.0
For 30 days	40	10.0	50	40.0

larvae after -5°C treatment; 58.1% of the unfed larvae was able to survive after exposure to -5°C for 10 days, but only 12.3% survived at -5°C for 30 days, and no unfed larvae survived at -5°C for 50 days.

The survival rate of unfed *I. persulcatus* larvae was high (83.8–100%) when they were exposed to -2 or -5°C for 10–30 days (Table 2). After exposure to -2 or -5°C for 50 days, however, the survival rate was reduced to 37.5–55.9%.

2) *Engorged larvae*. The percentage of molting of engorged *I. nipponensis* larvae was high (76.7–90.0%) when kept at 4°C for 10–50 days or -2°C for 10 days, but was reduced to 38.5–52.5% at -2°C for 20–50 days or -5°C for 10 days, and at -5°C for 20–30 days, only 5.6–10.0% of the engorged larvae molted to nymphs (Table 3).

The percentage of molting of engorged *I. persulcatus* larvae was relatively high even after -2 and -5°C treatments (Table 3). At -2°C for 20–50 days, for example, 64.3–

82.5% of the engorged larvae molted to nymphs, and even at -5°C for 20–30 days, 40.0–60.0% molted.

Tolerance of nymphs to low temperatures

1) *Unfed nymphs*. Both in *I. nipponensis* and in *I. persulcatus*, the unfed nymphs were tolerant to low temperatures (Table 4). The survival rates of the unfed nymphs of *I. nipponensis* and *I. persulcatus* were 93.3 and 100%, respectively, when they were exposed to -2°C for 50 days, and even after exposure to -5°C for 50 days, 78.6–80.0% of the unfed nymphs of both species survived.

2) *Engorged nymphs*. As shown in Table 5, the engorged nymphs of *I. nipponensis* successfully molted after 4 and -2°C treatments. The percentage of molting of the engorged nymphs was 77.8% even when kept at -2°C for 50 days. After -5°C treatment, however, the development of the engorged nymphs was seriously impaired; only 15.0–20.8% of the engorged nymphs

Table 4 Survival of unfed nymphs of *I. nipponensis* and *I. persulcatus* reared at 25°C and ca. 100% RH after the low temperature treatments at ca. 100% RH.

Duration of treatments	<i>I. nipponensis</i>		<i>I. persulcatus</i>	
	No. of unfed nymphs examined	Survived (%)	No. of unfed nymphs examined	Survived (%)
−2°C				
For 10 days	14	92.9	31	100
For 30 days	15	100	20	100
For 50 days	15	93.3	21	100
−5°C				
For 10 days	15	80.0	31	90.3
For 20 days	15	80.0	30	86.7
For 30 days	13	84.6	41	80.5
For 40 days	13	100	43	72.1
For 50 days	14	78.6	30	80.0

Table 5 Molting success of engorged nymphs of *I. nipponensis* and *I. persulcatus* reared at 25°C and ca. 100% RH after the low temperature treatments at ca. 100% RH.

Duration of treatments	<i>I. nipponensis</i>		<i>I. persulcatus</i>	
	No. of engorged nymphs examined	% molting	No. of engorged nymphs examined	% molting
Control (25°C)	19	89.5	54	79.6
4°C				
For 10 days	23	87.0	63	77.8
For 20 days	25	88.0	65	76.9
For 30 days	20	75.0	68	72.1
For 40 days	11	81.8	66	71.2
For 50 days	30	96.7	22	68.2
−2°C				
For 10 days	23	100	19	73.7
For 20 days	21	95.2	21	81.0
For 30 days	21	61.9	20	80.0
For 40 days	17	64.7	20	80.0
For 50 days	18	77.8	17	70.6
−5°C				
For 10 days	20	20.0	42	52.4
For 20 days	20	15.0	52	40.4
For 30 days	24	20.8	20	20.0

molted to adults after exposure to −5°C for 10–30 days.

The percentage of molting of engorged *I. persulcatus* nymphs was also high (68.2–

81.0%) after 4 and −2° treatments (Table 5). After exposure to −5°C for 10–30 days, however, the molting percentage was reduced to 20.0–52.4%.

DISCUSSION

Tolerance of eggs to low temperatures. The eggs of both *I. nipponensis* and *I. persulcatus* did not successfully develop after 4 and -2°C treatments. The low temperatures of 4 and -2°C are approximately the mean temperatures in January (the coldest month) in the low plains and mountainous regions (about 1,000 m above sea level) of Saitama Prefecture, respectively, according to the data from the Kumagaya local meteorological observatory. Thus, this result suggests that it is impossible for the eggs of both species to hibernate on the low plains and mountainous regions. However, the eggs of both species are scarcely exposed to low temperatures in winter because the adults are frequently observed on vegetation from spring through summer, but are scarce in autumn (Fujimoto *et al.*, 1987; Fujimoto and Yamaguti, 1990). Therefore, the low tolerances of eggs of both species to low temperatures do not have significant influence on their distribution patterns in the Chichibu Mountains.

Tolerance of larvae to low temperatures. Both in *I. nipponensis* and in *I. persulcatus*, the larvae were found on vegetation from spring through autumn (Fujimoto *et al.*, 1987; Uchikawa, 1993). It seems that the larvae of both species found in spring are mainly overwintering ones. On the other hand, a part of the larvae found in autumn hibernate in the engorged state. Thus, the unfed and fed larvae of both species are exposed to low temperatures in winter. The unfed and fed larvae of *I. nipponensis* showed an increased mortality with the duration of -2 and -5°C treatments. A temperature of -2°C is approximately equal to the mean temperature in January of the mountainous regions (about 1,000 m above sea level). In the mountainous regions of 1,000 m above sea level or more, therefore, the mortality of *I. nipponensis* larvae may increase in winter. On the other hand, the unfed and fed larvae of *I. persulcatus* were not seriously affected by low temperatures compared with

I. nipponensis. This suggests that the winter temperatures in the mountainous regions do not become an important mortality factor in *I. persulcatus* larvae.

Tolerance of nymphs to low temperatures. Both nymphs of *I. nipponensis* and *I. persulcatus* were abundant on vegetation in spring to summer, but scarce in autumn (Fujimoto *et al.*, 1987; Fujimoto and Yamaguti, 1990). On the other hand, the larvae of both species were collected from vegetation from spring through autumn (Fujimoto *et al.*, 1987; Uchikawa, 1993). Judging from these larval and nymphal activities of both species, it seems that most of the nymphs of both species hibernate in the unfed state. In this study, the survival of unfed nymphs of both species was hardly affected by being exposed to low temperatures. This high tolerance of unfed nymphs of both species to low temperatures makes it possible for them to survive in the mountainous regions where the winter climate is very harsh. Thus, this result does not fully explain why the distribution of *I. nipponensis* is limited in the lower montane zone.

The engorged nymphs of both species did not successfully molt, though the molting percentage of *I. nipponensis* was lower than that of *I. persulcatus*, when they were treated with -5°C . As previously stated, however, the mean temperature in winter of the mountainous regions is higher than -5°C . Furthermore, the nymphs of both species are rarely found on vegetation in autumn (Fujimoto *et al.*, 1987; Fujimoto and Yamaguti, 1990); *i.e.*, the engorged nymphs are scarcely exposed to low temperatures in winter. Thus, the tolerances of engorged nymphs of both species to low temperatures are not directly associated with their distribution patterns in the Chichibu Mountains.

ACKNOWLEDGEMENTS

I would like to thank Prof. E. Hori of the Laboratory of Medical Zoology, Saitama Medical School for his valuable comments on this study.

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摘 要

タネガタマダニとシュルツェマダニの耐寒性と秩父山系での分布の関係

タネガタマダニとシュルツェマダニの卵、幼虫、若虫を 10~50 日間、低温条件下 (4, -2, -5°C) に置き、その後、25°C に移して生存状況を観察した。両種の卵は、10~50 日間の 4°C あるいは -2°C 処理では、孵化率が 0~62.3% と低かった。両種の未吸血幼虫は、10日間の -2°C 処理では、すべて生存した。しかし、30~50 日間の -2°C 処理、あるいは 10~50 日間の -5°C 処理では、タネガタマダニの未吸血幼虫のほうがシュルツェマダニより生存率が低下した。両種の飽血幼虫の脱皮率は、10~50 日間の 4°C 処理では、76.7~93.3% と高かった。しかし、20~50 日間の -2°C あるいは 20~30 日間の -5°C 処理では、タネガタマダニの飽血幼虫のほうがシュルツェマダニより脱皮率が低下した。両種の未吸血若虫は、10~50 日間の -5°C 処理でも 72.1~100% の高い生存率を示した。両種の飽血若虫は、10~50 日間の 4°C あるいは -2°C 処理で、61.9~100% の高い脱皮率を示した。しかし、10~30 日間の -5°C 処理では、タネガタマダニの飽血若虫のほうがシュルツェマダニより脱皮率が低下した。シュルツェマダニの低温に対する高い耐性は、このダニが山地帯上部から亜高山帯にかけて分布できる理由をうまく説明する。しかし、タネガタマダニの分布が低山帯に限定される理由は、このダニの未吸血若虫が低温に対して高い耐性を示すために、低温耐性によつてうまく説明できない。