Host-seeking behavior of trombiculid mites on vegetation in relation to sika deer

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ABSTRACT  We collected larval trombiculid mites on vegetation monthly from October 1997 to February 2000, and from the heads of sika deer culled in March 2003 in Boso Peninsula, central Japan. Two species of trombiculid mites, *Neotrombicula nogamii* Takahashi, Takano, Misumi and Kikuchi and *Leptotrombidium scutellare* Nagayo, Miyagawa, Mitamura and Tamiya occurred on vegetation. Peak numbers of *N. nogamii* were found in January and *L. scutellare* numbers peaked in November. Both species were collected predominantly on the top of Sasa bamboo stems, where they formed clusters, though *N. nogamii* preferred heights of 40–50 cm. Furthermore, *N. nogamii* and *Walchia masoni* (Asanuma and Saito) were collected from deer. These findings indicate that vegetation is an important substrate for some trombiculid mites awaiting hosts.

KEY WORDS  chigger mite, host, aggregation, *Leptotrombidium scutellare*, *Neotrombicula nogamii*. *Walchia masoni*
Though scrub typhus is an endemic disease in Asia, the Pacific and the northeast coast of Australia, larvae of trombiculid mites are important vectors of the pathogenic rickettsia, *Orientia tsutsugamushi* (Traub and Wiseman 1974, Goddard 2000). Larval trombiculid mites are usually collected from the soil by Tullgren’s funnel method (Suzuki 1973, Uchikawa and Kumada 1978, Uchikawa et al. 1986) and/or from captured small mammals (Tamiya 1962, Nadchatram 1970). However, some species are collected from mid- and large-sized mammals (Kellogg et al. 1971, Pung et al. 1994, Forrester et al. 1996).

Host location and successful attachment is critical for the survival and reproduction of parasites. Though most ixodid ticks climb vegetation, clinging to the tips of stems or branches where they wait for direct contact with hosts, the final resting height is a major determinant of host specificity (Sonenshine 1993). Therefore, it may be advantageous for trombiculid mites that are parasites of mid- and large-sized mammals to await hosts on vegetation.

The trombiculid mite, *Neotrombicula nogamii* Takahashi, Takano, Misumi and Kikuchi, is reported as an ectoparasite of sika deer, *Cervus nippon* Temminck (Tamiya 1962, Takahashi et al. 2008), but is not found on rodents. We investigated host-seeking behavior of *N. nogamii* and other trombiculid mites on vegetation and infestation on deer in Boso Peninsula, central Japan.

**Materials and Methods**

**Sampling of mites on vegetation.** Sampling of trombiculid mites was carried out monthly from October 1997 to February 2000. Research was conducted in a forest gap on a plateau in the southern part of Boso Peninsula (Fig. 1A), which was the same area for collection of ticks by (Tsunoda 2007). Chinquapin pine, *Lithocarpus edulis*, and sugi cedar, *Cryptomeria japonica* surrounded the gap. The study area was covered with underbrush consisting of a bamboo (*Pteioblastus chino*), sedge (*Carex* sp.), eulalia (*Miscanthus sinensis*), hound berry (*Solanum nigrum*), bird’s tare (*Vicia cracca*), and kudzu-vine (*Pueraria lobata*). The study area of 12 x 28 m was divided into 21 plots of 4 x 4 m each (Fig. 1B). The plots were subdivided into 16 quadrats of 1 x 1 m each. One quadrat was selected in every plot by using a random number table on each sampling date. Three plants were chosen according to the cover rate of plant in each quadrat, resulting in a maximum of 63 (= 3 x 21) plants being chosen monthly. We recorded plant species, plant height, height and specific locale on the plant used by trombiculid mites, and the number of mites. Trombiculid mites found on plants were collected with a forceps between 1 p.m. to 5 p.m. Mites were mounted with Hoyer’s...
media (Krantz 1978) and identified under a microscope in the laboratory (Sasa 1956, Takahashi and Misumi 2007).

**Collection of mites from large- and mid-sized mammals.**  We inspected 24 head skins of sika deer, *Cervus nippon*, which had been culled in Boso Peninsula (Katsura 8; Otaki 3; Kimitsu 5; Ichihara 3; Kamogawa 5) in March 2003 (Fig. 1). After deer were shot, the head skins were removed from the animals and kept in a freezer until inspection.  We also inspected two Reeves’s muntjac, *Muntiacus reevesi* Ogilby, which had been culled in Kamogawa, Boso Peninsula in October 2001 and two raccoon dog, *Nyctereutes procyonoides* Gray, which were road kill in November 1995 and January 1999.

**Statistical analysis.** Chi square analysis was used to compare the heights of trombiculid mite collection on plants.  As expected frequencies of collected mites were < 5 in a height class, we grouped the height classes at both tails with the adjacent classes to create classes of adequate size.  Preference for specific plant locale was analyzed by Fisher’s exact test.  Stems were grouped with the stem top to create a class of adequate size.  The maximum height of a plant where mites were collected was compared with a questing height by Wilcoxon’s signed rank test.  The number of trombiculid mites composing a cluster was compared by Wilcoxon’s rank sum test with continuity correction.  Two-way ANOVA was used to analyze the relationship between mite species and site where deer were culled.  All statistical analysis was conducted by R software.

**Results**

**Trombiculid mites on plants.**  Two species of trombiculid mites were found on plants (175 *Leptotrombidium scutellare* Nagayo, Miyagawa, Mitamura and Tamiya, 216 *N. nogamii*).  *Leptotrombidium scutellare* appeared earlier in the year than *N. nogamii* (Fig. 2).  The number of *L. scutellare* peaked in November and *N. nogamii* in January.  Most mites were collected from a bamboo, *P. chino* (Fig. 3).  Dead branches of *L. edulis* and *C. japonica* were preferred next to (29% of *L. scutellare*, 9% of *N. nogamii*).  *L. scutellare* was collected mostly at 20-29 cm height although *N. nogamii* was at 40-49cm height (Fig. 4).  The maximum heights of plants where mites were collected were different from the questing height both in *L. scutellare* (*V*=0, *p*<0.05) and *N. nogamii* (*V*=276, *p*<0.01).  There was a significant difference in height of collection between *L. scutellare* and *N. nogamii* (*χ²*=105.12, d.f.=3, *p*<0.001).  More *N. nogamii* preferred the tops of stems than did *L. scutellare* (Fisher’s exact test, *p*=0.001) (Fig. 6).  *L. scutellare* and *N. nogamii* formed a cluster of their own species, although nine *N.
nogamii were found singly. More L. scutellare joined a cluster than did N. nogamii (W=92.5, p<0.05) (Fig. 7).

Attachment of trombiculid mites on middle and large sized mammals.

Twenty-one of 24 deer were hosted either N. nogamii or Walchia masoni (Asanuma and Saito). Both mites infested 11 deer. N. nogamii was found on 58% of deer and W. masoni on 75%. The number of mites per deer head was 15.1±7.1 for N. nogamii and 31.9±9.1 for W. masoni. Species composition of mites was variable between cull sites (Fig. 8). There was an interaction between mite species and sites (F=2.617, d.f.=9, 38, F=0.018).

Though L. scutellare was collected from the raccoon dog, W. masoni and N. nogamii were not (Table 1). However, W. masoni was collected from Reeves’s muntjac, and one L. scutellare was also collected.

Discussion

Our results show that both L. scutellare and N. nogamii preferred bamboo, specifically the stem tops, though the tendency was stronger in N. nogamii compared to L. scutellare. As bamboo leaves are one of preferred foods for sika deer in Boso Peninsula (Asada and Ochiai 1996), this site preference would result in higher mite/host contact. Our results also show that free-living N. nogamii were collected largely at heights of 40 - 50 cm. The height is similar to a species of tick, Haemaphysalis megaspinosa (Tsunoda and Mori 1995), that is a parasite of large mammals in Japan (Kitaoka et al. 1975). Furthermore, N. nogamii was collected from sika deer. These results suggest that N. nogamii awaits sika deer at a suitable site on plants.

On the contrary, we did not collect L. scutellare from sika deer. Our timing may have been too late for L. scutellare to be collected from sika deer, as mite numbers on vegetation peaked in November and deer-hunting season was February and March. In addition, L. scutellare may prefer small- and mid-size mammals (Sasa 1956, Tamiya 1962), instead of large mammals, as L. scutellare was collected from both raccoon dog and Reeves’s muntjac.

In three species of trombiculid mites we studied, only L. scutellare can transmit the tsutsugamushi pathogen in Japan. As the main host of the mite was thought to be rodents, unfed larvae were considered to transmit the pathogen to humans when they sit on the ground. However, our results suggest that L. scutellare can attack walking humans by contact with plants.

Some species of Leptotrombidium larvae tend to form a cluster close to the soil surface (Gentry et al. 1963). Cluster formation of those mites may result from egg
clusters from the same female. Though *L. scutellare* is known to form clusters on twigs and small rocks on the ground, this height is limited to within 10 cm of the ground (Ueno et al. 1955). However, our results showed that many *L. scutellare* were collected above 20 cm, which may increase the opportunity to attach mid-sized mammals. Although we did not collect free-living *W. masoni* on vegetation, this species is suspected to cluster, based on sampling by Tullgren’s funnel method (Uchikawa and Kumada 1978).

Since they form clusters, the spatial distribution of trombiculid mites is highly clumped, referred to as a ‘mite island’ (Traub and Wisseman 1974). Our results showed that *N. nogamii* and *L. scutellare* formed some clusters on plants. Sasa (1956) suggests that free-living larvae of trombiculid mites reduce the surface area of evaporation by forming a colony, i.e. cluster. Moisture retention by clustering has been demonstrated in adult American house dust mites, *Dermatophagoides farinae* Hughes (Glass et al. 1998). It is found that attached trombiculid mites tend to be closely grouped in clusters on the host with the mites all in the same degree of engorgement, indicating simultaneous attachment (Gentry et al.). Sasa (1956) also suggests that tight grouping of trombiculid mites by formation of a cluster increases the opportunity for greater number to parasitize simultaneously. Further studies are needed on clustering behavior so that the adaptive value can be understood.

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Figure legend

**Fig. 1.** Map of study sites. (A) The study site on Boso Peninsula, central Japan. Numbers show study sites in which deer were culled. Open star indicates the study site for collection of trombiculid mites on vegetation. (B) Hierarchical sampling design of the study site for collection of the mites.

**Fig. 2.** Seasonal abundance of *L. scutellare* and *N. nogamii* on plants from October 1997 to February 2000.

**Fig. 3.** Plant preference of two species of trombiculid mites, *L. scutellare* and *N. nogamii*. Number of the mites are shown on the top of graph.

**Fig. 4.** Height of trombiculid mites collected on plants. (A) *L. scutellare*, (B) *N. nogamii*.

**Fig. 5.** The maximum height and questing height of plants in which mites were collected. (A) *L. scutellare*, (B) *N. nogamii*.

**Fig. 6.** Parts of plants preferred by trombiculid mites.

**Fig. 7.** Number of trombiculid mites composing a cluster.

**Fig. 8.** Number of trombiculid mites, *L. scutellare* and *W. masoni* collected from sika deer captured in Katsuura, Otaki, Kimitsu, Kamogawa, and Ichihara.
Table 1. Trombiculid mites collected from raccoon dog, *N. procyonoides* Gray, and Reeves’s muntjac, *M. reevesi* Ogilby

<table>
<thead>
<tr>
<th>Host</th>
<th>Date</th>
<th>Site</th>
<th>No. <em>L. scutellare</em></th>
<th>No. <em>W. masonii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. procyonoides</em></td>
<td>Nov 1995</td>
<td>Otaki</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td><em>N. procyonoides</em></td>
<td>Jan 1999</td>
<td>Kamogawa</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td><em>M. reevesi</em></td>
<td>Oct 2001</td>
<td>Kamogawa</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td><em>M. reevesi</em></td>
<td>Oct 2001</td>
<td>Kamogawa</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td><em>M. reevesi</em></td>
<td>Oct 2001</td>
<td>Kamogawa</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Fig. 1.
Fig. 2.

Graph showing the number of miles per plant from 1997 to 2000 for two species:
- **L. scutellare** (dashed line)
- **N. nogamii** (solid line)

The graph indicates fluctuations in the number of miles per plant over the years.
Fig. 3.

- L. scutellare: n=175
  - 80% Sedge
  - 20% Dead branch (chinquapine pine and sugi cedar)
- N. nogamii: n=216
  - 100% Bamboo
Fig. 4.

(A)

Height (cm)

- 60<
- 50-59
- 40-49
- 30-39
- 20-29
- <20

Number of mites

(B)

Height (cm)

- 60<
- 50-59
- 40-49
- 30-39
- 20-29
- <20

Number of mites
Fig. 5.

A) *L. scutellare*

![Bar graph showing height (cm) for Maximum and Questing for L. scutellare.](image)

B) *N. nogamii*

![Bar graph showing height (cm) for Maximum and Questing for N. nogamii.](image)
Fig. 8.

The graph shows the number of mites per deer across different locations: Katsuura, Otaki, Kimitsu, Kamogawa, and Ichihara. The data is represented for two species: W. masoni (white bars) and N. nogamii (black bars). The error bars indicate the variability in the data.