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Title	Silk threads function as an 'adhesive cleaner' for nest space in a social spider mite
Author(s)	Kanazawa, Miki; Sahara, Ken; Saito, Yutaka
Citation	Proceedings of the Royal Society B : Biological Sciences, 278(1712), 1653-1660 <a href="https://doi.org/10.1098/rspb.2010.1761">https://doi.org/10.1098/rspb.2010.1761</a>
Issue Date	2011-06-07
Doc URL	<a href="https://hdl.handle.net/2115/45602">https://hdl.handle.net/2115/45602</a>
Type	journal article
File Information	PRSB278-1712_1653-1660.pdf



# **Silk threads function as an “adhesive cleaner” for nest-space in a social spider mite**

**Miki Kanazawa<sup>a</sup>, Ken Sahara<sup>b</sup> and Yutaka Saito<sup>a,\*</sup>**

<sup>a</sup>Laboratory of Animal Ecology, Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo, 060-8589, Hokkaido, Japan.

<sup>b</sup>Laboratory of Molecular Entomology, Research Institute of Agriculture, Hokkaido University, Kita-ku, Sapporo, 060-8589, Hokkaido, Japan.

\* **Author for correspondence** (yutsat@res.agr.hokudai.ac.jp).

## ***Manuscript information:***

17 text pages, 8 figures and 2 tables (text 5,022 words without Figures and Tables)

## ***Summary:***

Individuals of the social spider mite *Stigmaeopsis longus* live communally in narrow, humid nests made from silk threads and display nest sanitation behaviour through the coordinated deposition of faeces. We used artificial dust to experimentally determine that females of this species use silk threads to perform regular cleaning of the nest space and eggs. We first learned that silk-weaving behaviour is not a by-product of nest building (nest reinforcement); rather, it is actively performed as a function of cleaning the living space and eggs. Furthermore, we determined the effectiveness of the attending females by artificially manipulating their natural habitat, which is clearly connected to the cleaning behaviour by parental females. As such, we have uncovered an extraordinary new role of silk threads as devices for cleaning the nest space and/or eggs. These results strongly indicate that special adaptations for maintaining clean habitats are essential for animals to evolve aggregative social lives.

**Key words:** Spider mite, Tetranychidae, Sociality, Nest cleaning, Parental care

## Introduction

Group living in animals confers several advantages for improving individual lives, such as social organization and anti-predation effects (i.e. extended parental care and group defence (Taylor 1977; Calvert *et al.* 1979; Foster & Treherne 1981; Schmidt 1990).

Group living also involves inherent disadvantages, such as risks from mass disease infection, waste production and rapid resource deterioration (Schimid-Hempel 1988; Saito 2010). In relation to this, the importance of faecal load-reducing mechanisms (larval blind gut) in the evolution of eusociality has been recently discussed in Hymenoptera (Jackson and Hart 2009). A near total absence of larval faeces in the nests of social hymenoptera could facilitate sociality because of the reduced workload necessarily allotted to waste management. Human beings living gregariously in urban areas face a similar array of problems (Wolfe *et al.* 2007).

Among social insects such as earwigs, ants, and termites, there are several well-investigated examples where parental (or worker) care behaviour can successfully protect eggs against infection by fungi or microorganisms (Ramb 1976; Hughes *et al.* 2002). Female earwigs periodically secrete antibiotics from their mouthparts to sanitize egg surfaces. Without such care, eggs would seldom survive in humid habitats (Ramb 1976). Worker termites living in self-constructed nest chambers secrete saliva with antibiotic properties onto eggs, enhancing egg survival (Matsuura *et al.* 2000; 2007). As such, animals inhabiting humid and fungi/bacteria-abundant habitats, having more or less social and gregarious lifestyles can be expected to have evolved some specialized care behaviours for their offspring, especially eggs, to improve their survival. In other words, these reports strongly suggest that social and/or aggregative lifestyles are a prerequisite for maintaining clean living spaces.

A social spider mite species, *Stigmaeopsis longus* (Saito) (Acari:

Tetranychidae) uses dense silk threads to build nests and live gregariously over depressions in the host leaf undersurface (figure 1). Adults show strong counterattack behaviours against predators and often succeed in defending their nests and offspring by driving away or even killing nest intruders (Saito 1986). Furthermore, they display waste-management behaviours, i.e., they establish a “toilet” at their nest entrance and all nest members deposit faeces under rules realized through tactile and chemical cue (Sato *et al.* 2003). The waste-management behaviours of *S. longus* undoubtedly function to prevent nest space and food (leaf surface) from becoming fouled through group living. However, since the inside of the closed capsule-like nests are highly humid, some additional forms of nest-space cleaning behaviours are expected from *S. longus*.

We first determined that *S. longus* females ordinarily use silk threads to perform nest space/egg cleaning. These nest-cleaning behaviours are not a by-product of nest building (nest reinforcement), but are performed as a function of living space/egg cleaning. After confirmation of the above, we determined the effectiveness of the attending females in performing this behaviour by artificially manipulating their natural habitat.

## **2. MATERIALS AND METHODS**

The cooperative social spider mite species, *S. longus* occur naturally on *Sasa senanensis* Sieb. in a small wood in the campus of Hokkaido University, Sapporo, Hokkaido Japan. The mites used in the experiments were collected on 15, August, 2007. They were reared on undersurface of detached *S. senanensis* (collected from the same wood) in a climate-controlled chamber under  $25\pm 2^{\circ}\text{C}$ , 50-70% room humidity and photoperiod 15 : 9 (L : D) conditions. Laboratory experiments were carried out under the same temperature and humidity conditions other than photoperiod as mentioned later.

A detached leaf undersurface facing upward was used for the present experiments other than that conducted in field, because there were several difficulties to observe and to manipulate the leaf undersurface facing downward. Although mite researchers empirically know that such the reversed position of leaf affects little on mite life historical and behavioural performance (Yanagida et al. 2001), it is necessary to check the effects of such a reversed position if we have to give our special attention to the effects of gravity, such as cleaning behaviour. Thus we confirmed that any exuviae and most small pathogen-like particles (see later) that had been placed on leaf undersurface did not fall down if it was placed downward (Supplemental Data).

**(a) *What behaviours are used to clean a nest space?***

In a previous study, six typical behaviours were observed in *S. longus* females in their nests (Mori & Saito 2006): A. silk weaving, B. rest (including feeding), C. patrolling, D. wiggling, E. defecation, and F. oviposition. Among them, A, C and D behaviours were highlighted as candidates of female behaviours that might be related to the nest-space cleaning behaviour, because we often observed that numerous exuviae attached to the inner side of woven nest roof (ceiling) as shown in figure 1.

We first carried out an experiment to examine whether *S. longus* female cleans living space (leaf surface) when establishing its nest. We sprinkled globe-shaped silica particles (ca. 20  $\mu\text{m}$  in diameter, scastara<sup>®</sup>-red, Corefront Corporation, simply called as particles) on the leaf surfaces (ca. 70 particles/ $\text{mm}^2$ ), thereafter we individually introduced fertilized females and allowed to build new nests there. The adopted particle size, i.e. 20  $\mu\text{m}$  in diameter corresponds to the largest size of pathogen spore (the spore size varies 5  $\mu\text{m}$  to 20  $\mu\text{m}$  among pathogen species) and near the smallest size to observe under dissecting microscope. After the females started to build their nests, their behaviours were recorded for 12 h under continuous light conditions (ca. 800 fc) by a digital video cameras (Panasonic Digital time lapse AG-DTL 1H with camera,

Ikegami ICD-878<sup>®</sup>, hereafter called as VTR). The number of particles adhered to the web nest ceiling was counted at 3, 6, 9 and 12 h after the female began to make its nest web (the video recording was intercepted at each counting for 15-30 min necessary to observe the particles). If particles are picked up by certain behaviours, there must appear a correspondence between time-occupation (and also the number of silk-weaving behaviours ) and the number of particles on web ceiling. Then, time-occupation of each A-F behaviours and numbers of silk-weaving behaviours by the females during these intervals were counted by the use of the recorded video.

**(b) *Do mites clean nest by silk-weaving behaviour?***

Gravid females of *S. longus* at 3 days after maturity (mated) were individually allowed to construct nests on *S. senanensis* leaves. Three days after their introduction, we ripped about a half part of each nest web and sprinkled a number of particles through ripped opening (hereafter called hole) on the leaf surface within the nest. Then we counted the number of the sprinkled particles on the leaf surface inside the nests ( $n=18$ ). How many particles adhered to (beneath) the nest ceiling 24 h after the treatment was then counted. Simultaneously we recorded the mite behaviours for 24 h by VTR, and how many numbers of silk-weaving behaviours the female performed during the period by the use of the recorded video tape. As control experiments of the above experiment, we recorded female behaviour in nests with hole ( $n=9$ ) and in nests without hole ( $n=9$ ) by VTR.

Working hypothesis of this experiment was as follows: If mites behave to clean up nest space by silk-weaving behaviour, the frequency of the behaviour should increase with the number of particles sprinkled in nests, but if mites only behave to repair holed nests, the frequency of the behaviour should not increase with the number of particles. Then how many numbers of silk-weaving behaviours the female performed for the 24-h period was counted on the recorded

video.

**(c) *How long do silk threads remain adhesive?***

The observations of silk weaving behaviour suggested that silk threads being pulled out often attached particles, but those compositing web nest were not adhesive. Such that we expected that the silk thread quality changes with time. Then we determined how long the silk threads produced by *S. longus* females keep their adhesiveness. Twenty five females of *S. longus* were individually introduced onto new leaf arenas without nests and particles. We allowed them to spin threads for 5 min, thereafter removed the females. We sprinkled particles (ca. 70/mm<sup>2</sup>) on the leaf areas at different timing, i.e. 5 min ( $n = 7$ ), 1 h ( $n = 6$ ), 3 h ( $n = 6$ ) and 24 h ( $n = 6$ ) after the removal of females. Then we counted how many particles stuck to the silk threads after tapping the leaf surface with a shaft of fine tipped brush.

**(d) *Why are the eggs not cleared?***

If the silk threads produced by female frequently move the particles from the leaf surface to the ceiling of nest web (see results), we wondered why eggs deposited on the leaf surface inside nest were seldom swept by the threads (figure 1). Thus we hypothesized that there are some mechanisms that prevent *S. longus* eggs from adhering to the nest ceiling. One of the plausible modes was thought to be wiggling (behaviour D), i.e. the female behaviour on leaf surface just before and after oviposition.

In order to confirm the above, the gravid females of *S. longus* were individually introduced onto new leaf arenas and allowed to build web nests. Three days after the beginning of nest web building, we ripped about a half part of each nest web. Then the eggs in 16 nests were a little bit moved by fine tipped pin from the place where they were originally deposited. This treatment was to deactivate possible maternal cares on eggs. For the control, no manipulation was made to the eggs in 15 nests.

**(e) Does cleaning improve mite survival in the fields?**

From the above 4 series of experiments, it was indicated that *S. longus* females perform nest cleaning behaviour by silk threads (see results). If so, whether such a behaviour really functions in natural fields is an important question. However, there was a difficulty to evaluate directly the effect of nest cleaning behaviour on offspring survival in natural fields. Such that we observed the effect of female attendance under predator-free conditions on the survival rates of young in nests.

The field experiment was conducted during August 2008 in a small wood of deciduous trees with sub-canopy of *S. senanensis* in the campus of Hokkaido University, Sapporo, Japan. We first arbitrarily selected many *S. longus* nests each with a single female and 4 ~ 11 eggs on the leaves of *S. senanensis*. About 7 cm<sup>2</sup> leaf areas with single nests were surrounded with tangle-foot (sticky substance named Fuji tangle©, Fuji Yakuhin Kogyo Inc.) to repel all predators (this adhesive substance prevents predators from accessing prey nests, Mori *et al.* 1999). We settled 2 treatments as follows: Treatment 1 (female attending nest,  $n = 16$ ); we tapped nest roofs with a fine brush from outside, but did not remove the female. Treatment 2 (female removal nests,  $n = 17$ ); we tapped the nest web roofs with a fine brush from outside and drove the female away from the nest, then removed it. We recorded the number of eggs in each nest at the beginning of observation. Two weeks after the treatments, we evaluate survival rates of young. There was a difficulty to evaluate survival rates of young in treatment 1, because females living in the nests lay surplus eggs during 2 weeks. Since the eggs deposited at the beginning had developed to at least protonymph for two weeks, the survived immatures that developed from the eggs at the beginning of treatment 1 should be considered as survived protonymphs or more developed stages. Under this assumption, we calculated the immature survival rates in treatment 1 as “no. of survived immatures (protonymphs and more developed stages) at 2-week after” / “number of

eggs at the beginning of experiment”.

### **3. RESULTS**

#### **(a) *What represents cleaning behaviour?***

When introduced onto new leaf arenas, each *S. longus* female pressed its mouthparts (pedipalpi) onto one side of a depression on the undersurface of the host leaf (*S. senanensis*) and produce silk threads. The female then turned and walked straight to the other side of the depression (threads are extruded by the walking action, Saito 2010), where she pressed her silk production organs to the leaf once more. The female repeated this process many times. This series of behaviours corresponded to a single nest-building trial (Mori & Saito 2006), which we call “silk-weaving behaviour” (accurately spider mites simultaneously produce two threads that usually unite from paired silk-production organs, Jeppson *et al.* 1975; Saito 1977).

The *S. longus* females spent a considerable amount of time (up to 3 h) producing new nest webs during the early phases of nest construction. The greater the number of silk weaving behaviour, the greater the number of the silica particles (hereafter simply called as particles) raised up off the leaf surface (table 1). As a result, the leaf surface covered by the web nest became cleaner (i.e., the number of particles decreased as in figure 2). As most females rarely performed oviposition, wiggling, or patrolling behaviours during the 3-h nest construction period (table 1), it was apparent that the particles became attached to the woven nest roof (ceiling) of the web nests through silk-weaving behaviours.

#### **(b) *Is this really cleaning?***

However, doubt remained whether the mites perform the silk-weaving behaviour specifically for “nest-space cleaning” or for “nest-building only”. If the latter, the cleaning effect is simply a by-product. In well established nests with a hole, the number of particles attached to the ceiling of the web nest increased with the number of

particles initially sprinkled on the nest floor ( $r = 0.789$ ,  $P < 0.001$ ; figure 3). The number of particles attached to the ceiling of web nest increased with the number of silk-weaving behaviours ( $r = 0.773$ ,  $P = 0.0002$ ; figure 4). These results indicate that mites adjust their silk-weaving behaviours in response to the amount of dust in their nest space.

In the control experiments, a female performed ca. 386 weaving behaviours (= number of silk threads spun) in average in the nests with no particle and no hole. These were considered to be ordinal spinning behaviour in non-treated nests. Since ca. 496 weaving behaviours in average were observed in the nests with hole and without particles, 110 silk weaving behaviours were considered to be spun so as to repair the nest hole (there was a significant difference between no hole and hole on web,  $df = 1$ ,  $F$ -ratio = 21.25,  $P = 0.0003$  by Anova). That is, more weaving behaviours were observed in the presence of particles.

From figure 3, we calculated the regression line of the number of silk weaving behaviours ( $Y$ ) on the number of particles initially sprinkled ( $X$ ) as  $Y = 0.78X + 510.2$  ( $R^2 = 0.622$ ; intercept,  $P < 0.0001$ ; slope,  $P < 0.0001$ ). On this line, we obtained  $Y = 510.2$  (intercept) when  $X = 0$ . This value well corresponded to the value of 496 when only hole was made on nest web, stressing that surplus silk weaving behaviours observed under particle sprinkled conditions in figure 3 was caused by the existence of particles in nests.

**(c) *How long are silk threads adhesive?***

The threads produced by females were adhesive just after the production, but these became little adhesive with time. As shown in figure 5, ca. 20 particles stuck to the threads when the particles were sprinkled just after the production of threads (after 5 min and 1 h), but there were very small number of particles stuck to the web when the particles were sprinkled 3 h and 24 h after the production of threads. There were

significant differences between 5 min vs 3 h, 5 min vs 24 h, 1 h vs 3 h and 1 h vs 24 h, but insignificant differences between 5 min vs 1 h, and 3 h and 24 h by Scheffe's test (figure 5).

**(d) *Why are the eggs not cleared?***

The eggs of *S. longus* left intact in the nest never attached to the ceiling of the web nests, and those moved from the original depositing sites were almost all attached to the ceiling of web nests (figure 6). These observations suggested that there are some mechanisms that prevent eggs from being lifted up by the adhesive threads produced by females. We then observed the eggs deposited on the leaf surface within nest by an electron scanning microscope (SEM), and revealed that the eggs were deposited on web with a loose weave (figure 7, we called this web as web mat hereafter).

**(e) *Does cleaning gain mite survival in the field?***

The immature survival rate in the female attendance nests (treatment 1) was significantly higher than female removed nest (treatment 2 in figure 8). The survival rate at two weeks after the treatment 2 was 50%, but it was over 90% in treatment 1 (treatments 1 vs 2,  $P= 0.0008$  on the arcsine-root transformed values according to Mosteller & Youtz 1961). Thus female attendance significantly effective on the survival of offspring even under predator-free conditions in the fields was obvious.

#### **4. DISCUSSION**

Separating the nest-cleaning behaviour from nestreinforcement/repairing behaviour has been difficult because these are both commonly actualized by silk-weaving behaviour. We separated these by the use of artificial dust (silica particles) and nest manipulations. The results of experiment (a) indicated that *S. longus* females clean leaf surface through silk weaving behaviour when they establish their new nests. In experiment (b), silk weaving behaviour is actually enhanced when the nest space is dirty

(many particles). These results stress the reality of nest space cleaning behaviour in *S. longus*: If web spinning behaviour was performed only for nest repair and enforcement, there is no need for females to increase the frequency of such a behaviour with the number of particles in their nests. Therefore, it is concluded that the nest space cleaning is not a by product of nest repairing and/or reinforcement behaviour. It is apparent that the exuviae almost all attached to the ceiling of nest web in figure 1 are also caused by such a nest-cleaning behaviour.

The result of our subsequent experiment (§2c) is very suggestive. The threads spun by the females is adhesive, such that this adhesiveness is believed to function as adhesive tapes. The short-termed adhesiveness of threads after production must be advantageous as materials to make nest web solidier, because quick-drying is one of the convenient property of glue. Furthermore, if the adhesiveness of threads (web) would continue for a long time, it should make mites difficult to live within narrow space covered by such web, because *S. longus* need to touch their dorsal setae to the ceiling of web nest in order to recognize nest web existence (Saito 2010).

The result of experiment (d) showed that some protective means performed to the deposited eggs within nests prevent them from being lifted by silk weaving behaviour. By the SEM observation on *S. longus* eggs, we discovered that there is a web mat under each egg (figure 7, note that web covering eggs is often known in spider mites, but web mat under eggs is extraordinary, Saito 2010). Although direct observation is impossible, this mat seems to be formed by wiggling behaviour always observed just before and after oviposition (Mori & Saito 2006). Since the silk threads are adhesive 1 h after production, the eggs are fixed by such adhesive web mat on the leaf surface. Therefore, it may be concluded that *S. longus* females devise such mats to serve their eggs from being lifted up through the silk weaving behaviour. Several empirical observations suggested that eggs were attached accidentally to the ceiling of

web nest (eggs are kicked and moved by females) were wrapped by dense threads by repeated weaving behaviours and often become difficult to hatch. In other words, the existence of such a mechanism to avoid eggs from the dangerous weaving behaviour stressed that *S. longus* females perform cleaning of leaf surface inside nest. Furthermore, it is simultaneously suggested that silk-weaving behaviours also target egg surfaces for cleaning, because such behaviours must sweep up pathogen-containing dust from the egg surface as well as the leaf surface.

In the field experiment, we observed that the female attendance is effective on the survival of offspring without predation risk. Since many dead eggs and larvae in the non-female-attendance nests were colonized by fungi and/or microorganism hyphae (though it was difficult to know whether such organisms colonized before or after mite death), one of the factors of their mortality seems to be due to the infection of pathogen (Kikuchi & Tanaka 2010). As supposed from the laboratory experiments, *S. longus* females may clean their eggs as well as nest space. The positive effects of female attendance under predator-free conditions in the field may be caused by the nest/egg cleaning behaviour of females, although some other factors are not rejected in *sensu stricto*.

Meanwhile, two questions are arisen from the present results. One may concern the sources of dust in the densely covered nest space. *S. longus* has a special waste management behaviour, i.e. all nest members deposit them at a certain place (figure 1, Sato *et al.* 2003). This is realized by tactile and chemical cues and considered as one of nest sanitation modes by cooperation (Sato & Saito 2006). However, even if nest members keep to the regulation (actually they do so), this waste management adaptation may be not always perfect, because such a feces mass heaped on just outside nests easily infested by various fungi and bacteria (Kikuchi & Tanaka 2010). All nest members periodically go to the depositing place for defecation, such that it is inevitable

to take some spore and/or microorganisms on their legs and body from the feces mass. Because they move around the nest space after depositing feces, there is a great risk to propagate them over nest surface or on eggs. Exuviae with dust also dangerous waste for mites because they may carry pathogen, such that they must be managed by the nest cleaning behaviour as shown in figure 1.

The other question is concern the reason why mites attached dust and exuviae to the ceiling of the nest web. Fungus spore and bacteria attached on the ceiling of web nest is thought to be easy to dry up and become harmless in comparison with the leaf surface. Furthermore, repeated silk weaving behaviour must wrap them in the inner side of woven roof. Then danger of infection for mites living under the web seems to be well mitigated.

The discovery of communal sociality in a spider mite (Saito 1986), *S. longus*, tiny organism living on leaf surfaces and having very simple neural systems, was a topic in behavioural ecology (Yamamura 1987). Later, the details of sociality, the cooperative nest building and cooperative feces manipulation behaviours, have been continuously reported (Mori & Saito 2006; Sato *et al.* 2003). In this study, we discovered another kind of social behaviour in *S. longus*. The nest space and egg cleaning by silk threads is a new mode of social behaviour observed in the social spider mites. Self-produced silk threads are not tools in the strict sense of behavioural ecology, but we learn that *S. longus* use silk threads as if they were "device" of nest cleaning like diverting adhesive tapes for a sweeping.

Although silk threads have evolved independently in many different arthropods, (e.g., mites, spiders, moth larvae, Hymenoptera, and Psocoptera, Gerson 1985; Edgerly 1997) and may serve several functions such as providing nest (cocoon) material, prey-capturing devices, and lifelines, the nest-cleaning function observed in *S. longus* is the first such instance to be reported in the animal kingdom. Most spider mite

species, as can be imagined from their name, produce silk threads that function as basic lifelines for residing in arboreal habitats (Saito 2010). Such threads are sometimes used in contradictory ways to disperse from deteriorated habitats to new ones (Fleschner et al. 1956). Furthermore, several species use threads to construct web structures on leaf surfaces to prevent predation and mitigate adverse climatic conditions (Saito 1983). The most developed and sophisticated use of threads known in *Stigmaeopsis* species is as a material for constructing solid nests (Saito 2010). Here, we have demonstrated another marvellous use of silk threads as a device for nest space and/or egg cleaning. Such diversity in the usage of a single material strongly suggests that silk threads play a crucial role in the adaptive radiation of this taxon, Tetranychidae. Furthermore, the cooperative social living observed in *S. longus* must have evolved through silk threads that have the potential to be used in diverse ways as “devices” to establish aggregative and sedentary lifestyles.

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*Figure legends:*

Figure 1. Artificially removed web ceiling of a *Stigmaeopsis longus* nest with numerous exuviae attached to silk threads (micrograph taken in a scanning electron microscope).

E, egg; L, larva; exu, exuviae; F, female; fec, feces; M, male; N, nymph.

Figure 2. The process of nest-space cleaning by silk-weaving behaviours during the early phase of new nest construction. (a) Before female introduction. (b) Three hours after female introduction. (c) Web nest removal 5 days after female introduction. Note that most particles are attached to the nest ceiling and living floor is cleaned by a female silk-weaving behaviour. Artificial dust (particles) are “trapped” into the nest ceiling. The red particles are artificially sprinkled silica particles (ca. 20  $\mu\text{m}$  in diameter). Photographs were taken by dissecting microscope with digital camera.

Figure 3. Correlation between the number of silk-weaving behaviours and the amount of silica particles ( $\sim 20 \mu\text{m}$ ) sprinkled in the nest in experiment 2 ( $df = 1$ ,  $F$ -ratio = 26.40,  $r = 0.789$ ,  $P < 0.0001$  by ANOVA). The regression line of the number of silk weaving behaviours ( $Y$ ) on the number of particles initially sprinkled ( $X$ ) was  $Y = 0.78X + 510.2$  ( $R^2 = 0.622$ ; intercept,  $P < 0.0001$ ; slope,  $P < 0.0001$ ).

Figure 4. Correlation between the number of silk-weaving behaviours and the amount of silica particles swept up into the ceiling of the nest web in experiment 2 ( $df = 1$ ,  $F$ -ratio = 23.76,  $r = 0.773$  is significant at  $P = 0.0002$  by ANOVA).

Figure 5. Change in adhesiveness of silk threads with time. Vertical bars are standard deviations. There were significant differences (at least  $P < 0.006$  by Scheffe's test) between periods connected by line segments with alphabet a, but differences were insignificant between periods with b ( $P > 0.60$ ).

Figure 6. The effect of silk weaving behaviour on *Stigmaeopsis longus* eggs moved their original deposition places artificially. Moved ( $n = 16$ ): eggs were a little bit moved from

their original position, intact ( $n = 15$ ): egg were left intact on their original position. Vertical bars are standard errors. The actual number of eggs attached in "Intact" treatment was zero, but it is more than zero by the data transformation as follows. There was a significant difference between moved and intact treatments for the transformed values (for small samples) of attached rates ( $\theta(rad)$ ) by Mosteller & Youtz (1961) ( $df=1$ ,  $F$ -ratio=440.83,  $P<0.0001$  by ANOVA).

Figure 7. Web mat (thin threads on leaf surface) produced by females of *Stigmaeopsis longus* just before and after egg deposition (micrograph taken in a scanning electron microscope).

Figure 8. Survival rates of eggs in the nest with female and without female under predator-free conditions (by surrounding nests with Tanglefoot®). Treatment 1, female attending in nest; treatment 2, female removed. Vertical axis shows the transformed values (for small samples) of survival rates ( $\theta(rad)$ ) by Mosteller & Youtz (1961). Numerals and vertical bars in the figure are the number of replicates (nests) and the standard deviations, respectively. Statistics: female attendance v.s. female removed,  $P= 0.0008$  by Scheffe's test for the transformed values.

Table 1. Number of particles attached to web and time budget (%) for each behavior during 0-3, 3-6, 6-9, and 9-12 h periods after beginning of nest formation

	0-3 h	3-6 h	6-9 h	9-12 h
No. of particles attached	73.2	39.8	40.4	35.4
A. Silk weaving	77.2	27.3	35.5	19.1
B. Resting and feeding	21.9	58.2	57.4	67.4
C. Patrolling	0.9	14.4	6.9	13.1
D. Wiggling*	0.0	0.0	0.0	0.0
E. Faeces depositing	0.0	0.1	0.2	0.4
F. Oviposition	0.0	0.0	0.0	0.0

\* Wiggling that performed just before and after oviposition was not observed during 12 h, because females did not oviposit.

Table 2. Results of Scheffe's test for the adhesiveness of silk web in different periods after spinning.

Time after silk production	5 min	1 h	3 h	24 h
5 min	-	0.631	0.0002	0.0002
1 h	-	-	0.0044	0.0055
3 h	-	-	-	0.9997

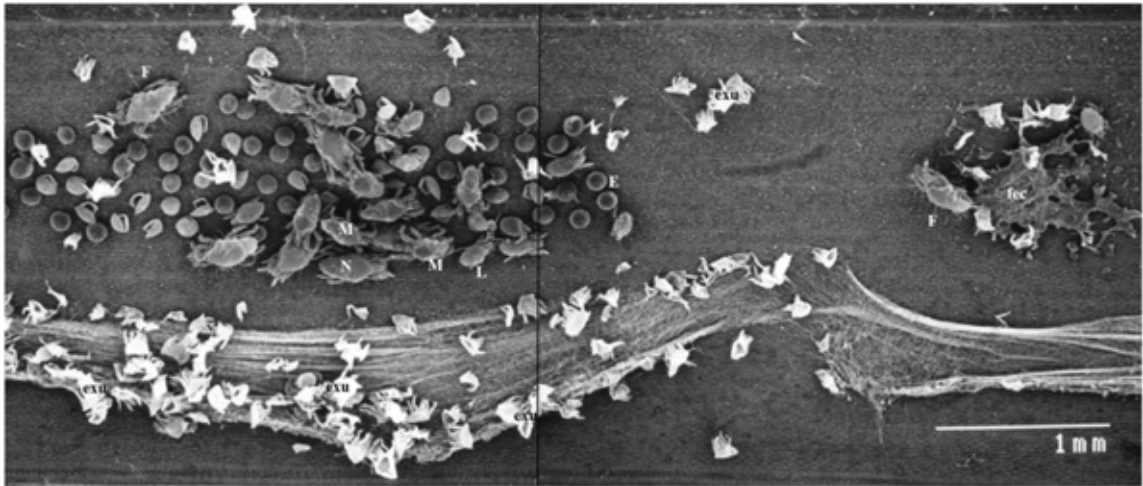


Fig. 1

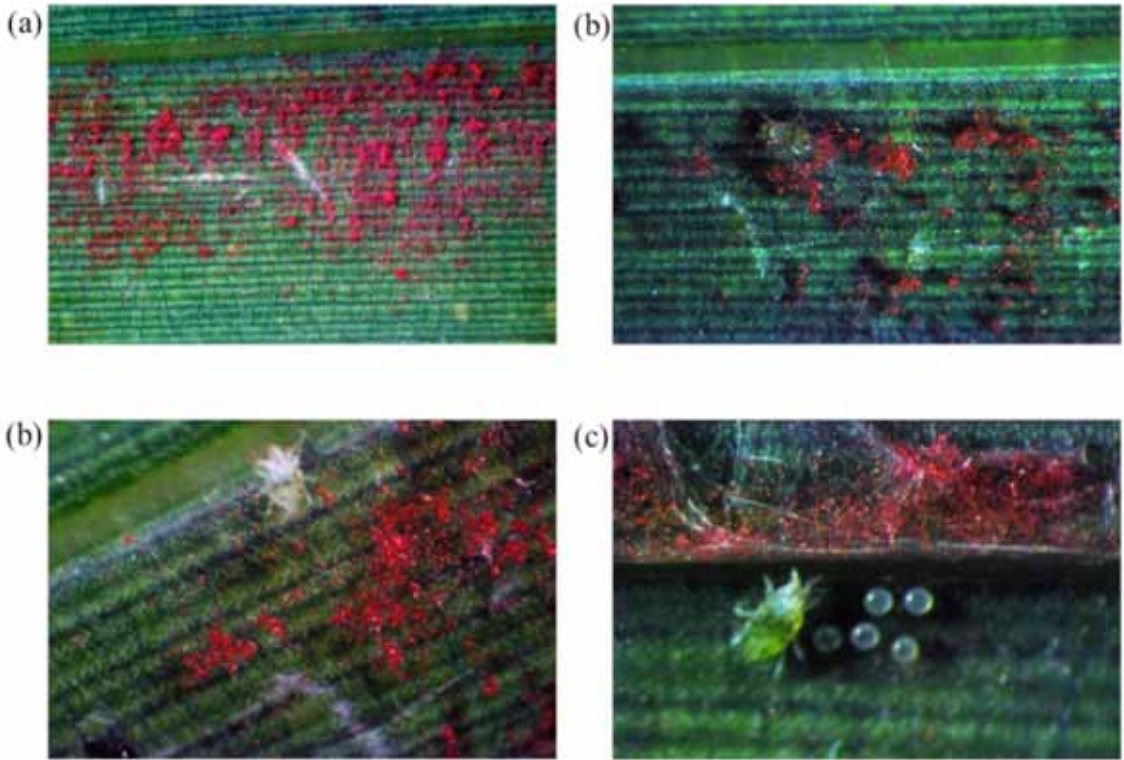


Fig. 2

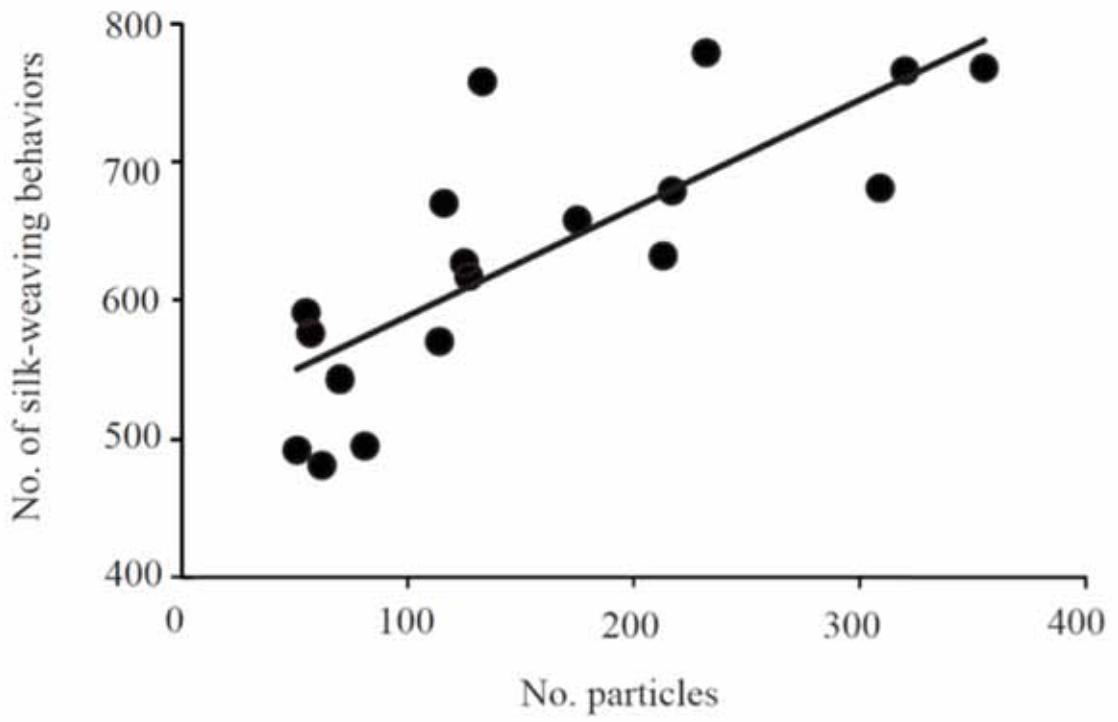


Fig. 3

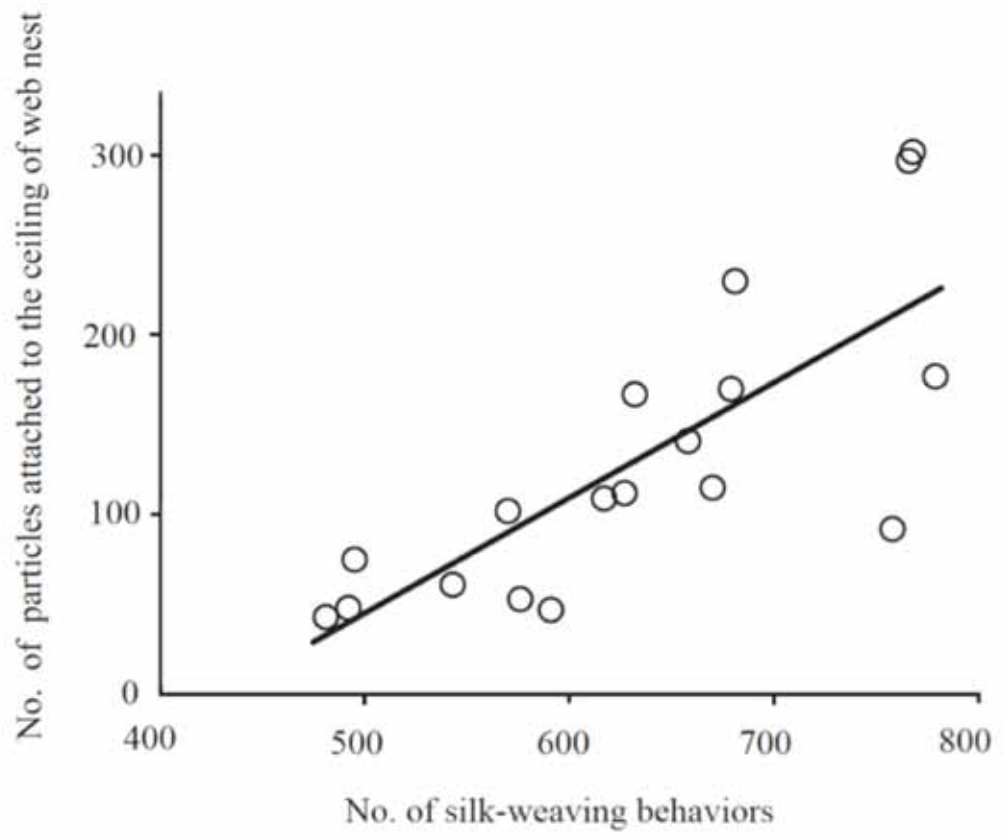


Fig. 4

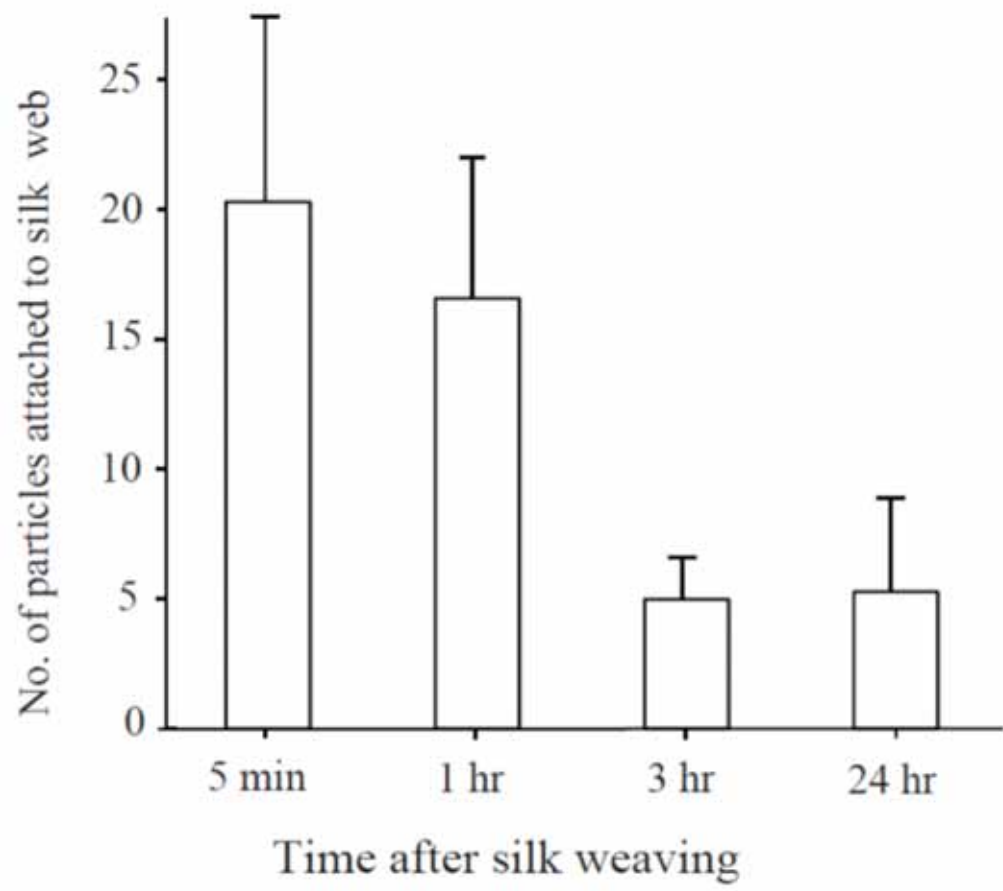


Fig. 5

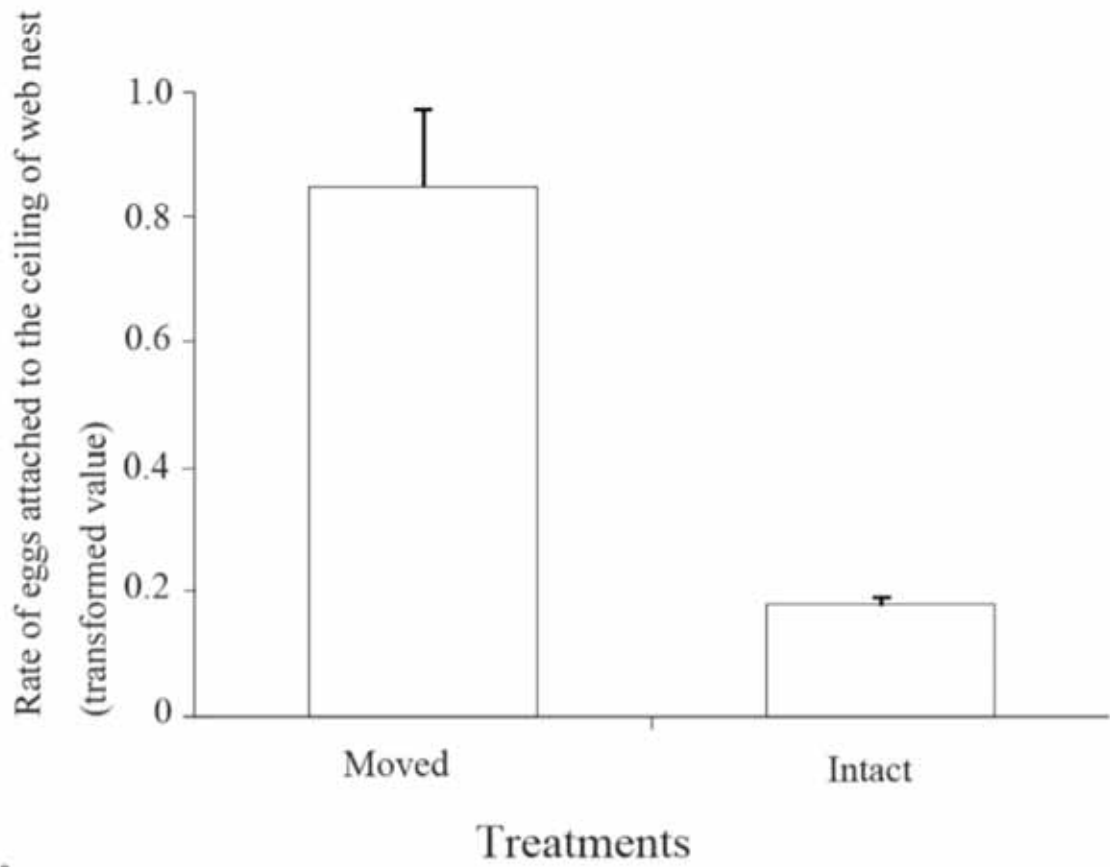


Fig. 6

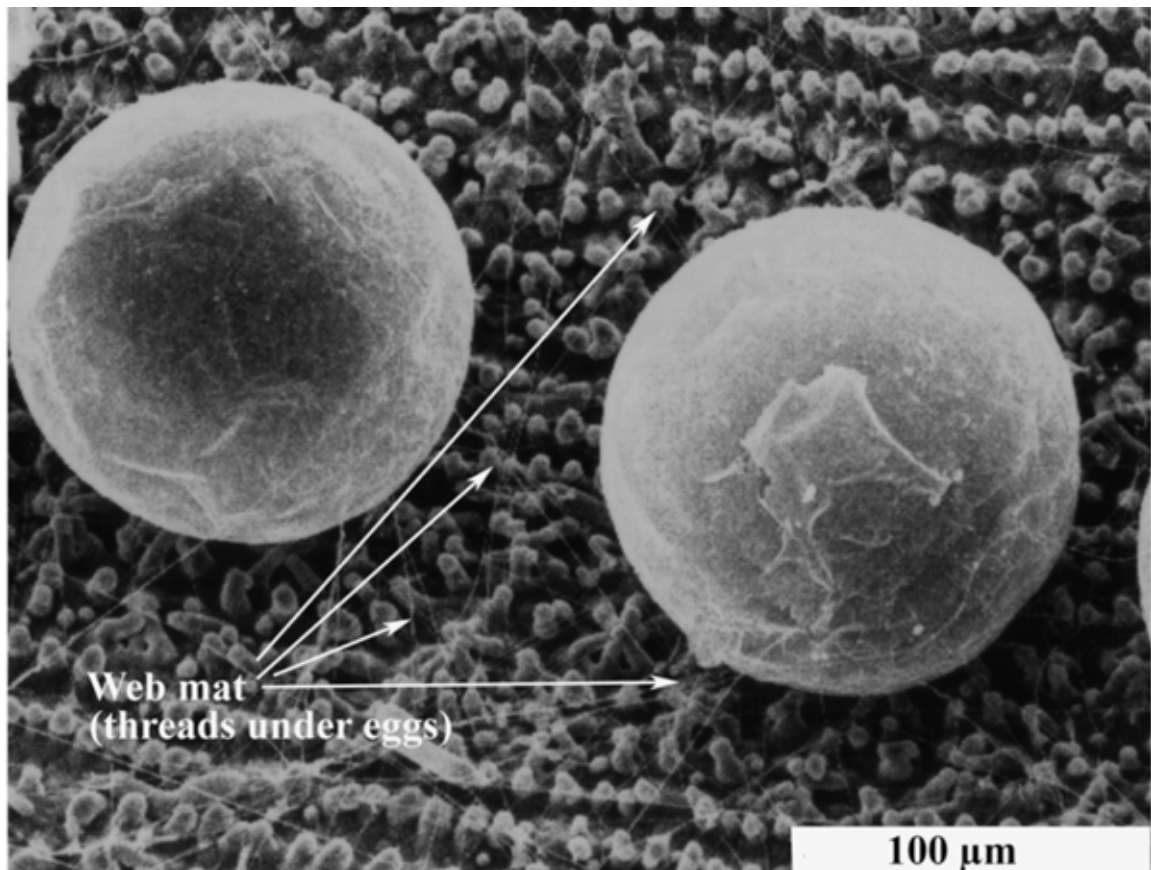


Fig. 7

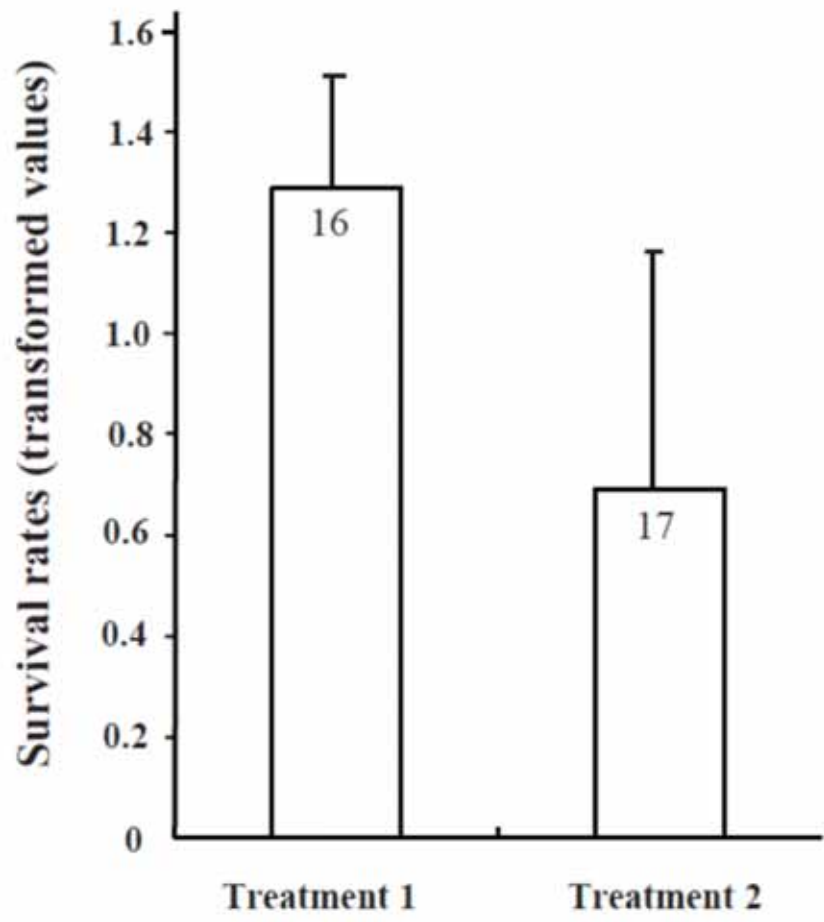


Fig. 8