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Variation in social behavior within a spider mite genus, *Stigmaeopsis* (Acari: Tetranychidae)

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Short title: social variation in spider mites

ABSTRACT

The spider mites belonging to the genus *Stigmaeopsis* construct extremely dense oval woven roofs (web-nests) over depressions on the lower surface of host leaves and are known to have a kind of sociality. The 4 species that occur on bamboo plants in Japan, show different nest areas. The nest area of *Stigmaeopsis longus* is the largest, followed by *S. celarius*, *S. takahashii* and *S. saharai* in decreasing order. Smaller nests effectively prevent adults of several predator mite species from intruding. We hypothesized that variation in nest size reflects different anti-intruder adaptations of this mite group in relation to their sociality. The larger nest makers may adopt an alternative anti-intruder strategy, i.e. counterattack by a large group, so as to compensate for the disadvantage of large nests. *S. longus* and *S. celarius* adults effectively defended their large nests against potential predators, and the effects of nest defense increased with the number of individuals in a nest. *S. takahashii* and *S. saharai* revealed no counterattack effect. Counterattack abilities that increase with the adult density and thus sociality may compensate for the vulnerability of larger nests.

Keywords: counterattack, group defense, sociality, cooperation, spider mites, web-nest

INTRODUCTION

Why there is diversity in sociality among nest-building invertebrates is an important question common to social organisms, because many social animals inhabit permanent or semi-permanent nests (e.g. Seger, 1991). Since nest structure determines the social unit, it also influences traits such as defensive behaviors (Alexander et al, 1991; Evans, 1977; Hansell, 1996; Lin and Michener, 1972; O'Donnell and Jeanne, 2002). Crespi (1994) suggests that one of the sufficient conditions for the evolution of eusociality (caste) is possession of shelter-like structures providing food. Indeed, among plant-parasitic arthropods, many aphids and thrips form various nest-like structures, "galls", and others include eusocial species that have specialized defenders against predatory or conspecific intruders (Crespi, 1992; Crespi and Mound, 1997; Foster and Northcott, 1994; Inbar, 1998; Stern and Foster, 1996, 1997). Variation in nest structure may therefore influence the diversity in social organization (Hansell, 1996).

In order to seek patterns of nest structure and social organization, comparisons of nest structures, behavior, social structures, and their functions among sibling species and/or among different forms in a species may be fruitful (Crespi and Choe, 1997; Dunn et al., 1998; Wcislo, 1997b; Wcislo and Danforth, 1997). These analyses may also contribute to how the difference in sociality is related to speciation, which is another important question of evolutionary biology (Hansell, 1996).

Spider mites are one of the simplest nest-building phytophagous organisms to varying in sociality (Saito, 1997). In *Stigmaeopsis longus* (Saito), females cooperatively build a large nest where adult males and females exhibit communal defense against predators. Several hundred individuals consisting of at least two generations of adults and immatures live gregariously over a long period (called "developed sub-sociality" in Saito, 1986a,b; "communal sociality" in Crespi and Choe, 1997). Another species with similar social behavior also occurs on Japanese pampas grass (Saito, 1990b). Many other species exhibit various kinds of social behaviors in this family (Saito, 1995); for example, females of several species build small nests and attend their offspring. Saito (1995) defined these as "sub-social" species.

Stigmaeopsis spider mites offer several advantages for the study of variation in social behavior; their host plants are very similar, some of them cohabit on the same host plants sympatrically (Saito and Takahashi, 1980; Takahashi, 1987), they are thought to be very closely related monophyletic species (Sakagami, 2002), and they are very easy to manipulate under experimental conditions. We have conducted a series of studies on this genus to determine 1) how sociality varies among sibling species in relation to their nest structures, and 2) what are the prime movers of evolution, and how the movers mold the variation in each sociality. In order to answer questions, we

previously analyzed variations in the nest structures and nests' abilities of prevention from predators' intrusion (Mori and Saito, in press). In the present report, we will analyze the counterattack behaviors of adults and discuss the questions based on the results obtained.

MATERIALS AND METHODS

Natural Histories of Mite Species

Stigmaeopsis mites construct extremely dense oval woven roofs (nests) over depressions on the lower surfaces of host leaves. They have a haplo-diploid genetic system (Saito, 1997). There are 4 species according to nest size (area); *S. longus* with the largest nest, followed by *S. celarius* Banks, *S. takahashii* Saito et Mori, and *S. saharai* Saito et Mori (Saito and Takahashi, 1980; Takahashi, 1987; Saito et al., 2004) all occurring on bamboo plants (*S. celarius* mainly occurs on *Phyllostachys pubescens* Manzel et Houzeau de Lehaie and the other three species on *Sasa senanensis* (Franchet et Savatier)). Under experimental conditions (25±1°C, 40-80% R.H. and a photoperiod of 15L-9D), the nest area (maximum length x maximum width) made by one female of each species over 48 h on the leaves of *S. senanensis* were *S. longus*=12.6 mm², *S. celarius*=6.9 mm², *S. takahashii*= 5.9 mm² and *S. saharai*= 4.2 mm². The mites of this genus are known to be monophyletic from ribosomal DNA sequences (Sakagami, 2002) but are reproductively isolated from each other (Saito and Takahashi, 1982; Mori and Saito, unpublished data). In *Stigmaeopsis* species, copulation occurs inside the nest, so that the difference in nest size may be crucial to their pre-copulatory reproductive isolation.

S. longus and *S. celarius* add continuously to their nests, so that the nest becomes larger as the group size increases. *S. longus* and *S. celarius* individuals usually live inside their natal web-nests throughout their lifetimes. In contrast, *S. takahashii* and *S. saharai* females repeatedly disperse to found new nests (Saito and Takahashi, 1982). Group size is approximately proportional to the nest size (Saito, 1990b; Mori and Saito, unpublished data): In the summer season (July to August), the average numbers of individuals of each species per nest (nests without predators) in the fields were *S. longus* male=2.31, female=6.31 (Sapporo, n=110 nests); *S. celarius* male=0.35, female=1.00 (Toyonaka, n=105); *S. takahashii* male=0.15, female=0.45 (Sapporo, n=350); and *S. saharai* male=0.19, female= 0.60 (Sapporo, n=74).

At least 7 spider mite species can occur on the leaves of *S. senanensis* in the field. Because of such abundant prey fauna, many species of phytoseiid and stigmaeid predators co-occur (Mori and Saito, in press). Several predators specialize on a single spider mite species (Chittenden, 2002; Chittenden and Saito, 2001; Saito, 1990a;

Yanagida et al., 2001). In a previous study (Mori and Saito, in press), we determined that nest size influences the probability of intrusion by predator species and that larger nests are less effective in preventing predators from intruding. Furthermore, the protective effect of the nest depends upon the predator species: Among 5 predator species that co-habit on *Sasa*, adult females of 3 predator species had great difficulty entering the nests of *S. takahashii* and *S. saharai*, but 2 other species could easily intrude and eat all 4 *Stigmaeopsis* species. One of the latter species, *Typhlodromus bambusae* Ehara, was used in the present study. *T. bambusae* can intrude into nests to feed upon all stages of all the 4 species of *Stigmaeopsis* (Mori and Saito, in press). Because all stages of *T. bambusae* are found exclusively inside the nests of *Stigmaeopsis* in natural forests (Saito, 1990a; Takahashi, 1987), it is considered to be a very specific predator of this genus. In the present paper, we concentrate limited predator-prey relationships between *Stigmaeopsis* mites and *T. bambusae* larvae out of the complex mite world on *Sasa*.

Materials

The mites used in this study were progeny obtained from field populations in Japan. *S. longus* and *S. takahashii* were collected on May 10, 1997, and June 15, 1998, respectively, from *S. senanensis* in Sapporo, Hokkaido (43°04' N, 141°20' E). These species were cultured on the leaves of *S. senanensis*. *S. saharai* was originated from individuals collected on June 9, 1998, from *Sasa kurilensis* (Ruprecht) in Higashikawa, Hokkaido (43°39' N, 142°48' E) and was also reared on the leaves of *S. senanensis*. *S. celarius* and the predator mite species, *T. bambusae* were obtained on March 30, 1998, from *P. pubescens* in Toyonaka, Osaka (34°46' N, 135°29' E). The *S. celarius* culture was maintained on the leaves of *S. kurilensis*. *T. bambusae* was cultured with spider mites arbitrarily selected from the stock cultures of *Stigmaeopsis* as prey on detached leaves of *S. senanensis*. All mites were reared under laboratory conditions (23±2°C, 40-80% R.H. and a photoperiod of 15L-9D). All experiments were conducted on the detached leaves of the same host plants cultivated in a greenhouse.

Comparison of counterattack efficiency among species

We measured the counterattack efficiency against the larva of the phytoseiid mite, *T. bambusae*. The larvae of this predator species never feed (Chittenden and Saito, 2001; Saito, 1990a), so that they are not active predators. However, the larvae molt to protonymphs without food at most within 2 days (Chittenden and Saito, 2001; Saito, 1990a), and Saito (1986a,b) demonstrated that the protonymph of *T. bambusae* actively feed on *S. longus* eggs and immatures. Because of this "forthcoming danger" posed by

T. bambusae larvae, Saito (1986a) showed that the counterattack behavior by adult males and females of *S. longus* must be regarded as biparental defense. Thus we regarded *T. bambusae* larvae as potential-predatory intruders (hereafter simply called as intruders). When *Stigmaeopsis* spider mites encounter *T. bambusae* larvae, they have 3 decisional options against the forthcoming danger: 1) they kill the larva, 2) they drive out the larva, or 3) they abandon their offspring and leave their nest.

To evaluate counterattack ability of two adult females or males, experiments were carried out on the underside of detached leaves. If we observe effective counterattack (explained below), such a phenomenon strongly suggests the existence of a kind of altruism due to alloparental care (Saito, 1986a). A leaf arena of 2 cm x 2 cm was prepared by surrounding it with water-soaked cotton. Two defender females (inseminated) and/or males picked up from each stock culture were introduced onto the experimental arena in the combinations shown in Table 1 ("initial conditions" of (1) to (5)) and kept under 23±1°C, 40-80% R.H. and a photoperiod of 15L-9D conditions for 2 days. After the defender females had constructed their nests and deposited several eggs, 5 experiments ("conditions for test" in Table 1) were started: A *T. bambusae* larva was placed on the surface of the nest web and its survival and location were periodically recorded (3, 6, 9, 12 and 24h after intruder introduction). Early work confirmed that the larvae of this predator species could immediately enter the nests of all the species without difficulty, so that the web size effect (i.e. prevention of intrusion by predatory intruders mentioned before; Mori and Saito, in press) could be disregarded. We regarded a counterattack as successful when the death of the *T. bambusae* larva was observed, or if the larva was observed outside the nest. Since leaving the nest is another reaction of adult spider mites (defenders), we also recorded their location.

Since the intruders of "(3) no defender adult vs. an intruder" in Table 1 never died regardless of defender species, those data are not shown in the comparison of "killing" effect against intruders (after 24 h). We calculated the "driving out" effect against intruders (after 24 h) as "proportion of intruders outside nest in each experimental group" - "proportion of intruders outside nest in control (no defender) group".

In addition to the above experiments, we evaluated the effects of the number of defenders per nest on counterattack success for species having the ability to counterattack by the same procedures ("experimental group" (6) to (9) in Table 1).

Encounter modes, behavioral patterns and consequences

Even if no successful counterattacks were observed in the experiment, we could not

conclude with certainty a species has no intruder-repelling behavior. Therefore, to examine in detail the anti-intruder behavior of all species, the encounter and subsequent interactions between defenders and *T. bambusae* larvae were observed.

A female and male of each species were introduced onto a detached *Sasa* leaf (1 cm x 1.5 cm) and kept under 25±1°C, 40-80% R.H. and a photoperiod of 15L-9D conditions for 2 days. A single defender of either sex and their eggs were left in the nest and the other defender was removed. One *T. bambusae* larva from a stock culture was introduced near the nest, and then the mites on the leaf were filmed by a video camera with a video cassette recorder (Victor Timelapse video cassette recorder BR-9000) in the daytime under the same conditions. The modes of encounter and behavior were recorded at most for 20 min after the first encounter between defender and intruder. If a certain outcome, i.e. “killing intruder”, “driving out intruder” or “deserting nest”, arose during this time, the observation was terminated.

All statistical analyses were performed by JMP statistical package (ver. 4.0.0, SAS Institute Inc.). All testing was applied under 2-tailed hypotheses and the significance level p was set at 0.05.

RESULTS

Comparison of counterattack efficiency among species

S. longus males, *S. longus* females, *S. celarius* males, *S. celarius* females and *S. saharai* males sometimes killed the intruder larvae (Figure 1a). Significant mortalities of 24 h after intruder introduction were observed in *S. longus* and *S. celarius* males. The results of Fisher’s exact probability tests of the mortality data (abbreviated hereafter as FEP) with sequential Bonferroni tests (Rice, 1989, abbreviated hereafter as SBT) within each species were as follows: *S. longus* males vs. control, $p < .0001^*$; *S. longus* females vs. control, $p = .49^{ns}$; *S. longus* males vs. *S. longus* females, $p < .0003^*$; *S. celarius* males vs. control, $p < .0001^*$; *S. celarius* females vs. control, $p = .04^{ns}$; *S. celarius* males vs. *S. celarius* females, $p = .04^{ns}$ (p -values show results of FEP, * is significant and ns is not significant at $p = .05$ after corrections by SBT). No effective counterattack by killing was observed in either sex of the *S. takahashii* or *S. saharai* (FEP; all combinations: $p > .49$).

To examine differences in mortality of predator along with the time between *S. longus* and *S. celarius* males, Kaplan-Meier survival analysis between the survival curves of attacked intruder larvae was conducted (Figure 2). There was a significant difference between the 2 survival curves (Wilcoxon test, *S. longus* male vs. *S. celarius* male: $\chi^2 = 4.49$, $p = .03$), showing that *S. longus* males had a more effective “killing” effect than *S. celarius* males.

A significant counterattack success by “driving out” 24 h after intruder introduction was observed in *S. longus* males, but not in *S. longus* females (Figure 1b, by FEP with SBT within the *S. longus*: *S. longus* males vs. control: $p=.002^*$; *S. longus* females vs. control: $p=.35^{ns}$). The other species did not show any significantly effective “driving out” results (by FEP, $p>.11$).

Defender’s reaction to intruder

One day after the *T. bambusae* larva introduction, two defender reactions to the intruder were observed: “staying inside nest” and “staying outside nest” (Figure 3). Both sexes of *S. longus* tended to stay regardless of the presence of the intruder. *S. celarius* males also tended to stay regardless of the presence of the intruder, whereas 73.9 % of *S. celarius* females left their nests in response to the intruder (all *S. celarius* females stayed their nests when there was no intruder). Furthermore, most *S. takahashii* and *S. saharai* adults, regardless of sex, left their nests 24 h after the intruder introduction.

Group effects in defense of individuals of the same sex and/or opposite sexes

Next, we observed whether there was some kind of group effect of individuals (the effects of the number of defenders per nest on counterattack success) either same sex or different sex on the counterattack success.

Counterattack experiments for several adult combinations were conducted ((6), (7), (8) and (9) in Table 1). The results are shown in Figure 4. Logistic regression analyses for each species showed that there are group effects in killing and driving out against the intruder larvae in *S. longus* males, although *S. longus* females did not show any effective group effect (Table 2). Two males of *S. celarius* also killed the intruder larvae, although no group effect between males in “driving out” was detected (Table 2). *S. celarius* females showed a group effect in driving out, but the coefficient of interaction between *S. celarius* male(s) and female(s) in “killing” was significant (Table 2) suggesting that males and females seem to disturb each other when they live together.

Encounter modes, behavioral patterns and consequences

In all experiments, regardless of defender sex, defenders were frequently observed to zigzag within their nests (called “patrolling” in Saito, 1986a) just after the first encounter with an intruder. Thereafter, the defender repeatedly encountered the intruder. We observed three encounter modes <"encounter" doubled>: mode 1: defender approached resting intruder, mode 2: defender approached moving intruder and mode

3: resting defender met moving intruder. Encounter mode 1 was observed most frequently among the three modes in all cases, i.e. *S. longus* males: 7/15(mode 1 frequency / total frequency); *S. longus* females: 20/29; *S. celarius* males: 11/11; *S. celarius* females: 21/34; *S. takahashii* females: 36/48; *S. saharai* males: 27/36; *S. saharai* females: 22/29, except for *S. takahashii* males for which mode 2 was most frequent (17/29). “Mode 1 + mode 2” (defender encountered intruder) were more frequent than “mode 3” (intruder encountered defender) in all species and sexes (binominal test, $p < .01$) except for *S. celarius* females ($p = .06$). FEP tests with SBT between species within each sex were applied to the data for “mode 1 + mode 2” and “mode 3”. We found no significant differences in the frequencies between species. These results suggest that defender mites first discovered the intrusion of *T. bambusae* larva.

The behavioral modes of the defenders after the encounters with intruders greatly varied among species and sexes. We observed 8 behavioral patterns in the defenders (Saito, 1986a):

Capturing (Mode A): the defender captures the intruder with its front pair of legs and pushes its mouthparts against the intruder’s body.

Direct pursuit (Mode B): the defender chases after the intruder.

Repeated jabbing, (Mode C): the defender thrusts out its front pair legs and repeatedly dashes its body against the intruder.

Beating or drawing, (Mode D): the defender raises its first pair legs and hits the intruder.

Avoiding, (Mode E1): the defender avoids contact with the intruder.

Escaping, (Mode E2): the defender runs away from the encounter site.

Disregarding (Mode F1): there is no visible behavior between individuals.

Tapping. (Mode F2): the defender taps the intruder with legs.

Modes A to D are regarded as aggressive, modes E1 and E2 as timid, and modes F1 and F2 as neutral behaviors. Assuming that behavioral aggressiveness decreases in order of A, B, C and D, the frequencies of male aggressive behavior tended to decrease with the order of nest size, i.e. *S. longus*, *S. celarius*, *S. takahashii* and *S. saharai*. In fact, mode A (“capturing”) was only observed in *S. longus* males, and mode B (“direct pursuit”) was often observed in *S. longus* and *S. celarius* males. The *S. takahashii* and *S. saharai* males sometimes exhibited modes C and D, but they never showed modes A and B (Figure 5). Furthermore, *S. takahashii* and *S. saharai* males frequently performed mode E2 (“escaping”).

The behavior of defender females also differed among species. *S. longus* females were the most aggressive (Figure 5), frequently performing modes B (“direct

pursuit”) and C (“repeated jabbing”), but exhibiting mode E2 (“escaping”) at a low frequency. *S. celarius* females showed modes C (“repeated jabbing”) or E2 (“escaping”) at similar high frequencies. *S. takahashii* and *S. saharai* females also showed mode C (“repeated jabbing”), while mode E2 (“escaping”) was predominant in *S. saharai* females.

To examine the differences in frequencies of the behavior between species within each sex, chi-square tests were applied to the combined data (categorizing aggressive, timid and neutral behaviors) (Table 3). In males, the behavior patterns of *S. longus* and *S. celarius* were different from those of *S. saharai* probably because of aggressive behaviors (Figure 5). In females, although the patterns of all four species were similar, almost all the combinations were significantly different, but *S. longus* and *S. takahashii* were not different.

DISCUSSION

Variation in social behavior

There is great variation in the counterattack success (Figure 1) as well as in the behavior against intruders (Figures 3 and 5) among the 4 species of *Stigmaeopsis*. Furthermore, the effectiveness of counterattack behavior tended to increase with nest size. This may mean that sociality also varies among the species. From the present results and previous information about age structure in the nest (Mori, unpublished data; Saito, 1986a), we confirmed that there are 2 communal-social species (*S. longus* and *S. celarius*) and 2 sub-social species (*S. takahashii* and *S. saharai*) on bamboo plants in Japan (Saito, 1995).

Alternative anti-predator strategies related to nest-size variation

The genus *Stigmaeopsis* in which 7 social and sub-social species are included has been reinstated by Saito et al. (2004) (all type specimens are deposited in the Hokkaido University Museum and several reference specimens will be deposited in the Smithsonian National Museum of Natural History). Before Saito (1990c), *Schizotetranychus celarius* was the sole representative of this group, with several forms discriminated using the different lengths of several dorsal setae (some of these setae function as sensors to recognize the existence of the nest, Saito and Takahashi, 1982). Since these species can be assumed to be monophyletic and very close sibling species from a molecular phylogeny (Sakagami, 2002), we are able to explore the reasons why sociality has diversified in these species.

One of the explanations of the wide variation in this genus is that they adopt two alternative strategies against predatory intruders: “prevention of intrusion by smaller

nests” in *S. takahashii* and *S. saharai*, and “defense by many individuals in larger nests” in *S. longus* and *S. celarius*. On *Sasa* bamboo, the main host of *S. longus*, *S. takahashii* and *S. saharai*, at least 5 predatory mite species (including *T. bambusae*) can potentially feed on the species group (all can eat the eggs of all spider mite species, if there is no web-nest, Mori and Saito, in press). Experimental studies indicated that *S. takahashii* and *S. saharai* nests effectively prevent 3 of these predators from intruding, but *S. longus* nests exclude only one species (Mori et al., 1999; Mori and Saito, in press).

Although *S. longus* nests can exclude only a single predator species, their large sizes permit many individuals to live for a long time and thereby increase the counterattack success for nest defense when attacked by predators. In the present study, 2 individuals of the same sex were more successful than one in “killing” or “driving out” in both *S. longus* and *S. celarius* (Figure 4). The result in *S. longus* is in agreement with Saito (1986b), suggesting that group living confers an advantage upon these 2 species. Furthermore, a positive density effect, i.e. "cooperation" in the sense of Dugatkin (1997) occurs between adult males (Figure 4), even though these species have male-haploidy (Hamilton, 1972; Saito, 1986b). Large nests as well as the low probability of male dispersal (Mori, unpublished data) may guarantee the viscosity of individuals in time and space, and this may be leading to kinship within nests reflected in the peculiar mildness of the male-male relationships (Saito, 1987; 1997) among spider mites in which males usually have acute rivalry (Potter et al., 1976; Saito, 1990b).

The smaller nests of *S. takahashii* and *S. saharai*, on the other hand, cannot contain the necessary number of adults required to gain defensive success by counterattack, so that leaving the nest may be their only option (Figure 3), if predators invade their nest-webs. Although predator-prey interactions are generally hypothesized to influence many aspects of biology including social behavior (Alexander et al, 1991; Evans, 1977; Lin and Michener, 1972), there are few examples showing that the nest influences the variation of social behavior (Hansell, 1996; O'Donnell and Jeanne, 2002; Wcislo, 1997a).

Other unsolved problems

We could not detect any positive group defense between sexes in either the *S. longus* and *S. celarius* (Figure 4 and Table 2), though Saito (1986b) suggested such cooperation in *S. longus*. The difference may result from male-female partnership difference in the experimental design of Saito (1986b)'s and the present study. In Saito (1986b)'s experiments, *S. longus* females and males in a nest were actually mated

partners, whereas in the present study, inseminated females and males were taken from mass stock cultures. Whether this difference in partnership (thus in kinship between actors and recipients) can explain the different results between these two studies should be tested in the future.

In this study, we have only focused on the interactions between adult spider mites and the larvae of a single predator species. However, the interactions between spider mites and their predator mites are much more complex, because they may vary with prey and predator stages (Saito, 1986b). Furthermore, it is difficult to say that there is no counterattack ability (thus social behavior) against other predator species in *S. takahashii* and *S. saharai*, both of which showed no counterattack success against *T. bambusae* larvae in this study. The discovery of aggressive behavior (Figure 5) in these species suggests rather that they can counterattack effectively against different species and/or stages of predatory intruders, even though the behavior is only effective for a short period of nest life.

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Figure legends

Figure 1

Comparison of counterattack efficiency among the 4 species. 2MA: 2 males, 2FA: 2 females, respectively. a) Comparison of "killing" effect against intruders among the 4 species (after 24h). b) Comparison of "driving out" effect against intruders among the 4 species (after 24h).

Figure 2

Kaplan-Meier survival curves of the intruder in experimental groups of *S. longus* males or *S. celarius* males vs. intruder.

Figure 3

Comparisons of "reaction of defender against intruder" among the 4 species (after 24h). 2MA: 2 males, 2FA: 2 females. Open bars are the results of 2 defenders vs. intruder. Solid bars are the results of control experiments (2 defenders vs. no intruder). P-values over bars are the results of the Fisher exact tests.

Figure 4

Comparisons of efficiency of group defense between *S. longus* and *S. celarius*. MA: male(s), FA: female(s). a) Comparison of "killing" effect against intruder among different defender combinations (24h). b) Comparison of "driving out" effect against intruder among different defender combinations (24h). Vertical axis is the same as that of Figure 1b).

Figure 5

Behavior frequencies of the defender against the intruder. Behavioral patterns are in accordance with Saito (1986a) (Detailed explanations appear in the text): mode A: capturing, mode B: direct pursuit, mode C: repeated jabbing, mode D: beating or drawing, mode E1: avoiding, mode E2: escaping, mode F1: disregarding and mode F2: tapping. Solid, shaded and open bars indicate aggressive, timid and neutral behaviors, respectively.

Table 1 Experimental design of counterattack effects

Experimental group	No. of replicates ^a				Initial conditions		Conditions for test			
	SL	SC	ST	SS	Male	Female	Male	Female	Eggs	Intruder
(1) 2 males vs. Intruder	27	28	25	25	2	2	2	0	+	1
(2) 2 females vs. Intruder	26	23	24	26	0	2	0	2	+	1
(3) Control (No defender vs. Intruder)	25	26	24	24	0	2	0	0	+	1
(4) Control (2 males vs. No intruder)	27	26	27	26	2	2	2	0	+	0
(5) Control (2 females vs. No intruder)	26	28	30	26	0	2	0	2	+	0
(6) 1 male vs. Intruder	20	19	-	-	2	2	1	0	+	1
(7) 1 female vs. Intruder	19	19	-	-	0	2	0	1	+	1
(8) 1 male & 1 female vs. Intruder	18	18	-	-	2	2	1	1	+	1
(9) 2 males & 2 females vs. Intruder	18	21	-	-	2	2	2	2	+	1

^aSL: *S. longus*, SC: *S. celarius*, ST: *S. takahashii*, SS: *S. saharai*.

Table 2 Results of logistic regression analyses for group defense*

S. longus

Source	Killing			Driving out		
	Estimate	χ^2	<i>p</i>	Estimate	χ^2	<i>p</i>
Male	1.73	37.70	<.0001	-1.12	19.29	<.0001
Female	-0.19	0.44	.505	-0.36	1.85	.174
Male*Female	-0.26	0.73	.394	0.03	0.01	.912

S. celarius

Source	Killing			Driving out		
	Estimate	χ^2	<i>p</i>	Estimate	χ^2	<i>p</i>
Male	1.07	12.27	.001	-0.81	1.67	.196
Female	0.19	0.37	.546	-1.38	5.41	.020
Male*Female	-0.84	7.01	.008	0.23	0.15	.699

*The model of logistic regression is $y = \beta_0 + \beta_1 \text{Male} + \beta_2 \text{Female} + \beta_3 \text{Male*Female}$, where domains of Male and Female are 0, 1 and 2.

Table 3 Results of chi-square tests applied to combined frequencies of behavioral modes (aggressive, timid and neutral behaviors in Figure 5) between species

<I think decimal fraction=3 enough other than 0.0006 or 0.0001>

Combination [*]	p-values of chi-square test	
	Male	Female
SL vs SC	n.a.	<.0001 [‡]
SL vs ST	.035	.280
SL vs SS	.002 [†]	.003 [‡]
SC vs ST	.036	.0006 [‡]
SC vs SS	.011 [†]	.002 [‡]
ST vs SS	.523	.009 [‡]

^{*}: SL: *S. longus*, SC: *S. celarius*, ST: *S. takashii*, SS: *S. saharai*.

n.a.: Chi-square test was not applicable.

[†] and [‡]: significant at $p=.05$ after correction by sequential Bonferroni

Figure 1

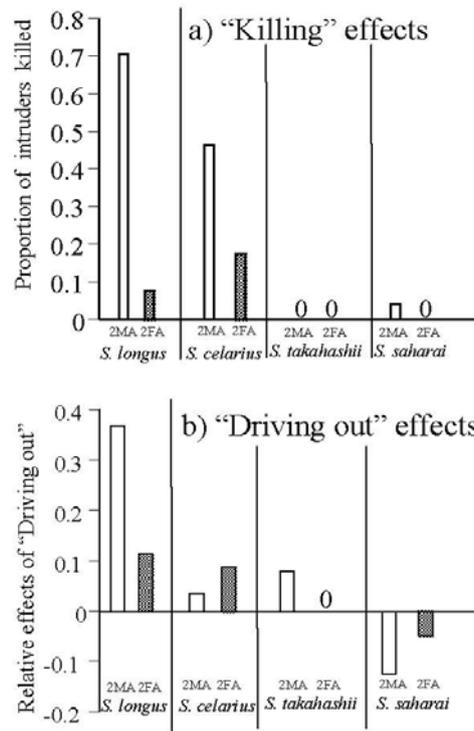


Figure 2

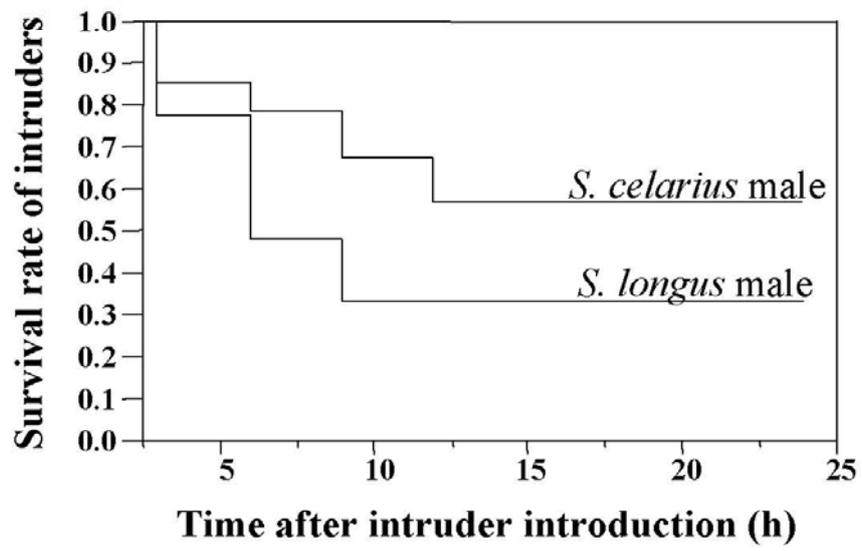


Figure 3

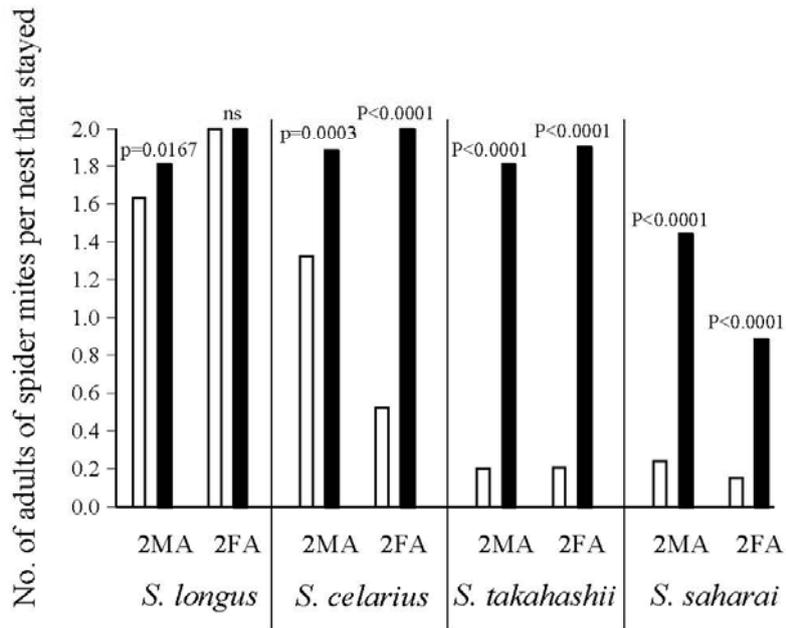


Figure 4

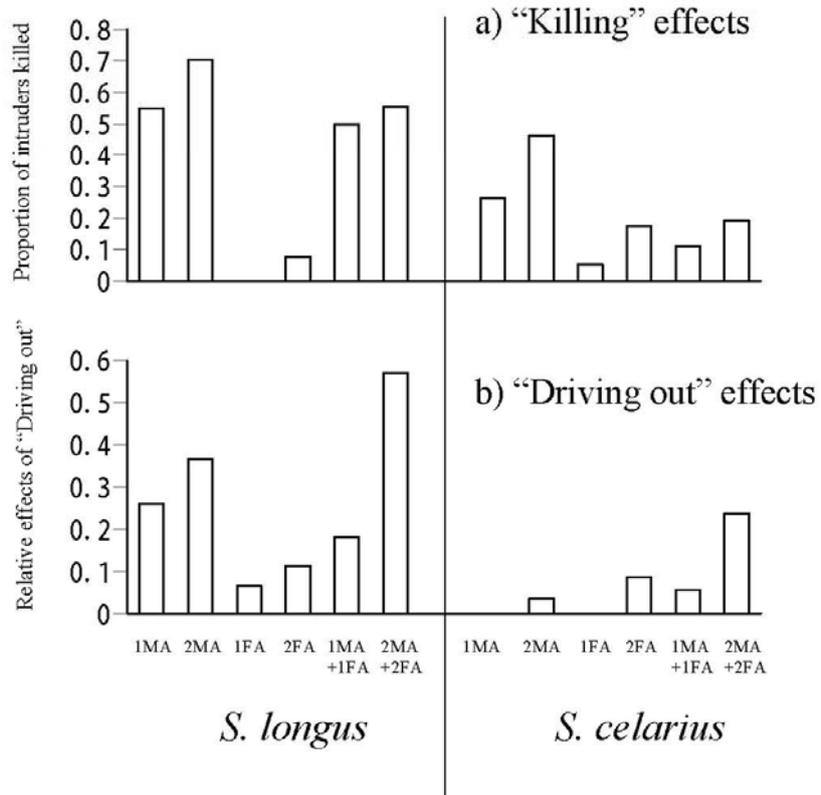


Figure 5

