



RESEARCH ARTICLE - WASPS

Levels of Parasitism by *Xenos oxyodontes* (Strepsiptera, Stylopidae) among Adult Hornets and Its Voltinism in the Host *Vespa analis* (Hymenoptera, Vespidae)

SHUN'ICHI MAKINO

Department of Forest Entomology, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, Japan

Article History

Edited by

Alexandre Somavilla, INPA, Brazil

Received 10 September 2024

Initial acceptance 17 October 2024


Final acceptance 05 December 2024

Publication date 30 January 2025

Keywords

Social wasp, parasitism, stylopization, life cycle.

Corresponding author

Shun'ichi Makino 

Department of Forest Entomology,
Forestry and Forest Products Research
Institute, Tsukuba, Ibaraki, 305-8687 Japan.
E-Mail: shunichimakino@gmail.com

Abstract

Styloped adults of *Vespa analis* in collected nests and bait-trapped samples were examined to learn the intensity of parasitism and the life cycle of the parasite *Xenos oxyodontes*. Of the 48 nests collected between June and October in central Japan, 60% contained styloped adults, with 8.2% of the total intranidal adult population styloped. Stylopedization rates varied by sex and caste, accounting for 7.3% in workers, 12.3% in males, and 1.7% in new queens. The overall sex ratio (male ratio) of the parasite was 0.76 within adult hornets, consistently male-biased in both the month of collection and the sex of the host. Styloped adults occurred even in nests collected in October, toward the end of the nesting season. Considering the developmental periods of both the host and the parasite, such *Xenos* individuals found in the late-season nests belonged to the second generation rather than the first generation (descendants of overwintered females). Adults of *V. analis* bait-trapped between May and September consistently included hornets infested with *X. oxyodontes* females carrying first instar larvae, indicating that parasite infection occurs through this period. Female parasites found on workers produced in the current year were observed to carry first instar larvae from August onwards. This observation would be impossible if *X. oxyodontes* were univoltine and newly emerged females always overwintered without releasing larvae within the same year. Thus, the results clearly confirm the bivoltine life cycle of *X. oxyodontes* in the study region.

Introduction

Vespine wasps play significant roles in human welfare and well-being. While their stings can lead to severe health issues, including fatalities due to anaphylactic reactions (Otaki, 2005; Perkins & Yates, 2017), they also act as predators of various agricultural and sanitary pests and as facultative pollinators for certain plants, thereby providing important regulatory ecosystem services (Brock et al., 2021). Given their ecological and economic significance, it is essential to understand how their abundance and colony productivity are affected by various factors, including natural enemies.

Nests of vespine wasps are not immune to threats from organisms that negatively affect them. Various natural

enemies are associated with these nests, from birds and mammals that destroy nests and consume immatures to microorganisms that infest nest inhabitants (Edwards, 1980; Matsuura & Yamane, 1990; Schmid-Hempel, 1998). Some insect enemies do not directly invade the nests but instead enter them by phoresy, in which first instars attach themselves to host wasps that subsequently transport them to the nest, as seen in strepsipterans of the genus *Xenos* that parasitize social wasps (Matsuura & Yamane, 1990; Hughes et al., 2003; Beani et al., 2018; Kathirithamby, 2009).

Five of the 33 accepted species of *Xenos* have been recorded from the hornet genus *Vespa* (Benda et al., 2022). Of the five species, two occur in Japan: *Xenos moutoni* Buysson and *Xenos oxyodontes* Nakase & Kato (Nakase & Kato, 2013;



Kanzaki et al., 2023; Kudô et al., 2024a). Information on the prevalence (parasitic levels) of these parasites in their host hornets has been obtained from various regions of Japan, mainly by collecting hornets using bait traps (Makino & Yamashita, 1998; Makino, 2001; Sayama, 2012; Kanzaki et al., 2023; Oyaizu & Kudô, 2013; Kudô et al., 2024a, b). These studies have reported the proportions of parasitized (styloped) hornets in different castes and sexes and seasonal variations in parasitism levels in field-collected adults. However, our understanding of the levels of stylopedization (parasitism by strepsipteran insects) within and between nests is limited (Matsuura & Yamane, 1990; Kifune & Arakawa, 1996; Makino et al., 2009), although this information is crucial for assessing the impact of these parasites on the development and productivity of host colonies. Furthermore, some bionomic details of these parasites are still lacking, such as the timing of entry of first-instar larvae into nests and voltinism (number of generations per year). Although it has been suggested that *X. moutoni* and *X. oxyodontes* are multivoltine, that is, have more than one generation per year (Matsuura & Yamane, 1990; Matsuura, 1995; Kudô et al., 2024b), it has not been proven yet.

This study has two main objectives. The first is to investigate the levels of stylopedization by *X. oxyodontes* among adult hornets in nests of *Vespa analis* Fabricius in Japan. The second objective is to learn the timing of first-instar parasite infection within the nesting season and to estimate the voltinism of the parasite.

For the first objective, the percentage of stylopedized adults, the sex ratio of the parasite, and the caste and sex of the hosts were examined using nests collected at different times during the nesting season. For the second objective, stylopedized hornets were bait-collected in the same area where nests were collected to observe how the proportion of female parasites containing first-instar larvae changes over the season. Because strepsipteran first instars are short-lived in general (Maeta & Kifune, 1990; Kathirithamby, 2009), the period when female parasites carry first-instar larvae ready to be released is reasonably considered as the period of infection of the host by the larvae. These data on seasonal changes in stylopedization levels in nests and the percentage of female parasites harboring first-instar larvae will contribute to a better understanding of the biology of *X. oxyodontes*, including its voltinism.

Materials and Methods

Species studied: *Xenos oxyodontes* had previously been referred to as "*X. moutoni*" until it was described as a distinct, cryptic species (Nakase & Kato, 2013). *X. oxyodontes* primarily parasitizes *Vespa analis*, while *X. moutoni* targets other *Vespa* species (Nakase & Kato, 2013; Kanzaki et al., 2023). According to Matsuura and Yamane (1990), first-instar larvae of *X. moutoni* attach to foraging spring queens or workers in

the field, who then bring the larvae back to their nests. Once inside, the larvae enter the larval body of the host and develop without killing it. The fully developed parasites of both sexes eventually leave the nest with the adult hosts. Although wasps parasitized by strepsipterans are not killed, they usually lose their ability to work or reproduce (Matsuura & Yamane, 1990). In this sense, the strepsipterans are virtually parasitoids rather than true parasites (Kathirithamby, 2009), though the effect on male hosts is only subtle in *Polistes* (Cappa et al., 2014; Beani et al., 2017).

Vespa analis is a common hornet in Japan, constructing aerial nests on various artificial structures or tree branches. In central Japan, nests are initiated by overwintered, inseminated females (gynes) in early May. Workers begin to emerge in late June, and the colony terminates in November after producing reproductive adults (Matsuura & Yamane, 1990). Although usual workers die before winter, workers parasitized by female *Xenos* often overwinter in rotten wood, as do gynes (Matsuura & Yamane, 1990; Tatsuta & Makino, 2003).

Study area: Nest collection and bait trapping were carried out in two neighboring cities, Tsukuba (36°05'N, 140°04'E) and Ushiku (35°58'N, 140°08'E), located in Ibaraki Prefecture, Honshu, Japan. These cities lie within the Kanto Plain, where the climate is temperate. In Tsukuba, the mean annual temperature between 1991 and 2010 was 13.8 °C, with an average monthly maximum of 25.5°C in August and a minimum of 2.7 °C in January (Japan Meteorological Agency, 2024).

Nest collection: Post-emergence nests of *V. analis* were collected between June and October from 1998 to 2003 within a 10 × 10 km area encompassing Tsukuba and Ushiku. The number of nests collected each year varied from one to 16. These nests, typically located 0.5–5 m above the ground, were primarily found on houses or in hedges within residential areas. Before collection, the entrance hole on the nest envelope was plugged with a cotton ball soaked in ether to anesthetize the adult hornets inside. If nests were collected during the day, returning hornets were captured with an insect net for 0.5–1 hour after nest removal to gather as many adults as possible.

The collected nests were dissected in the laboratory, and all adults were examined for sex, caste, and stylopedization by *X. oxyodontes*. Stylopedized individuals were identified by observing a male puparium or a female cephalothorax protruding between the gastral segments (Makino & Yamashita, 1998). The sex and number of parasites were recorded for each stylopedized wasp, along with the caste and sex of the host. Data from all collected nests were pooled for analysis.

Bait trapping: Adult *V. analis* were collected using bait traps from April to November between 1998 and 2003. The bait consisted of a mixture of sweet potato liquor (25% alcohol) and an equal amount of orange juice (Makino, 2001). Clear plastic beverage bottles (30 cm high, 10 cm wide, 2,000 ml in volume) with a 3 × 3 cm window in the upper part were used as bait containers.

The traps were set at five locations: 1) a nursery at the Forestry and Forest Products Research Institute, where small willows (*Salix* spp.) were planted; 2) a small coppice primarily composed of oaks (*Quercus serrata* Thunb. and *Q. acutissima* Carruth.); 3) trails in Takasaki Nature Park, which featured small to medium-sized broad-leaved trees including *Q. serrata*, *Q. acutissima*, and *Castanea crenata* Siebold et Zucc.; 4) an apiary at the National Agricultural and Food Research Organization, where 10–15 beehives were maintained; and 5) trails in Ushiku Nature Park, which had vegetation similar to site 3. Sites 1–4 were located in Tsukuba and 5 in Ushiku, all within the same 10 × 10 km area where the study nests were collected, and were 2–6 km apart from each other.

Ten traps were each installed in the above sites except site 4, where 16 were set. The traps were installed at 10–50 m intervals and tied to trees approximately 1.5 m above the ground. The traps were checked approximately every two weeks to replenish bait and collect trapped hornets. The collected hornets were preserved in 70–80% ethanol for later examination. Some of the materials collected between 1998 and 2000 were used in previous studies on *Xenos* parasitism (Makino, 2001) and vespine species compositions (Makino & Sayama, 2005).

Stylopized hornets collected by the bait traps were examined for the number and sex of the parasites, the emergence of male parasites indicated by empty puparia, and the presence of first-instar larvae in the cephalothorax of female parasites. If female parasites contained first-instar larvae in the cephalothorax, they were considered ready to release the larvae or in the process of doing so, as reported in the stylopid *Pseudoxenos iwatai* Esaki, which parasitizes eumenine wasps (Maeta, 1963a).

Estimation of the timing of infection and voltinism: Female *X. oxyodontes* overwinter within the bodies of female hornets (Matsuura & Yamane, 1990). Overwintered females include both queens and workers, as workers parasitized by *Xenos* females frequently overwinter (Matsuura & Yamane, 1990; Tatsuta & Makino, 2003). *Xenos* females release first-instar larvae after their hosts awaken from overwintering (Matsuura & Yamane, 1990; Makino, 2001). If *X. oxyodontes* is univoltine, these first instars should develop into adults that would overwinter, if female, without producing larvae in the same year. Conversely, if the species is multivoltine, the female offspring of overwintered parasites should produce larvae that mature and mate within the year. Therefore, if female parasites on current-year hosts carry first-instar larvae, it is likely that *X. oxyodontes* is at least bivoltine in the study area.

Results

1) Stylopized adults in the nests

A total of 48 post-emergence nests of *Vespa analis* were collected over six years, from 1998 to 2003 (Supplementary File 1). Of these, 29 nests (60%) were parasitized, containing at least one stylopized adult hornet (Supplementary File 2). The percentage of parasitized nests increased as the season progressed, peaking in September before decreasing in October (Fig 1). Nests containing reproductive adults first appeared in July, with their proportion increasing toward the end of the nesting season. Nearly all reproductive adults were males; new queens were found in only two nests collected in October. Across all nests, the percentage of stylopized hornets was 8.2% when all castes and sexes were combined, with 7.4% in workers and 12.3% in males. Two of the 18 new queens (11%) were stylopized from the nests containing new queens.

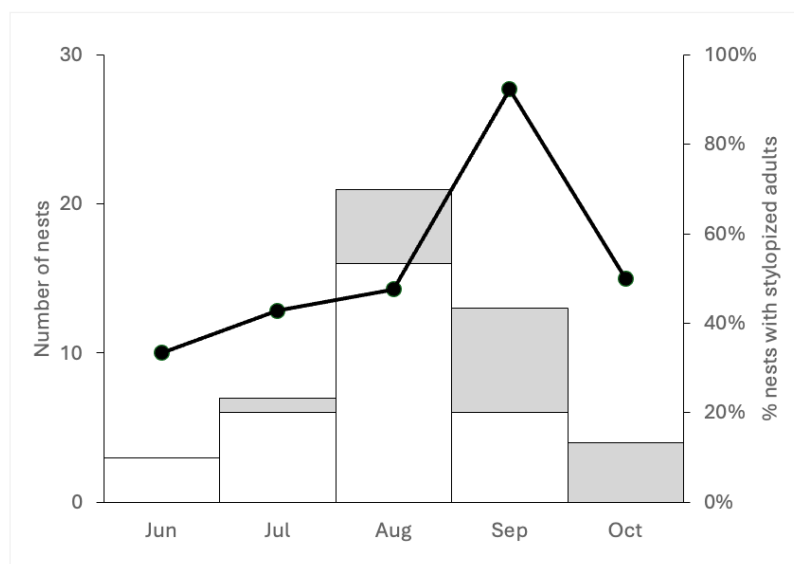


Fig 1. Monthly number of *Vespa analis* nests collected (columns) and the percentage of parasitized nests among them (dots and lines). Parasitized nests contained at least one adult hornet stylopized by *Xenos oxyodontes*. The gray and white portions of the columns represent nests with and without reproductive adults (males and/or new queens), respectively.

Monthly changes in the number and percentage of stylopized individuals among intranidal adult hornets are shown separately for workers and males in Fig 2. The earliest stylopized adults, which were all workers, appeared in late June when the nests contained only a small number of workers. The first stylopized males appeared in August. In both workers and males, the percentage of stylopized adults was highest in September, 11.0% in workers and 25.6% in males. Even in October, late in the nesting season, nests still contained 2.5% and 2.8% stylopized individuals in workers and males, respectively.

The number of parasites per host ranged from 1 to 13, although most hosts (75% of workers and 58% of males) were monoparasitized (Fig 3; Supplementary File 2). Among the superparasitized hosts, 25% contained male and female parasites, observed in both workers and males. No male

puparia had yet produced adults, and no female parasites contained first-instar larvae in the intranidal hosts.

The overall sex ratio of the parasites (the number of males divided by the total number of parasites across all hosts) in the intranidal adult hornets was 0.76, significantly different from 0.5 (χ^2 test; $p < 0.001$). When calculated separately for workers (0.74) and males (0.82), the sex ratio remained male-biased. The two stylopized new queens hosted only female parasites (Supplementary File 2). The deviation from a sex ratio of 0.5 was significant in both workers and males (χ^2 test; $p \ll 0.001$). The monthly sex ratio was also male-biased from June to September, being significantly different from 0.5 in August (0.79; $p < 0.01$) and September (0.77; $p \ll 0.001$). In October, females slightly outnumbered males, though the sex ratio (0.44) was not significantly different from 0.5 ($p > 0.05$).

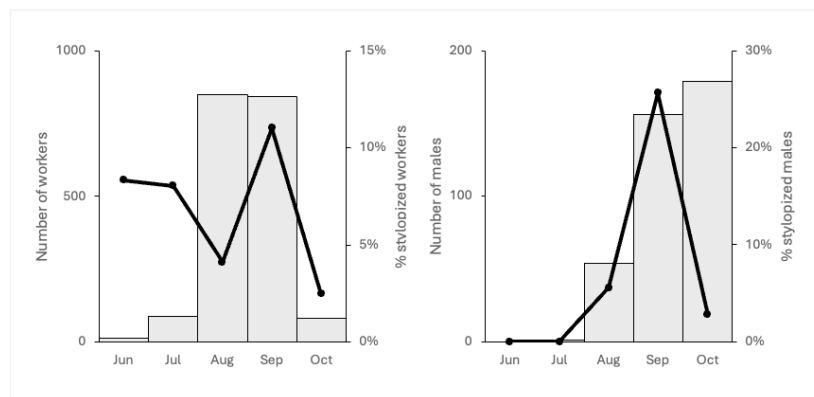


Fig 2. Monthly number of adult hornets in *Vespa analis* nests (columns) and the percentage of individuals stylopized by *Xenos oxyodontes* (dots and lines). Left: workers. Right: males.

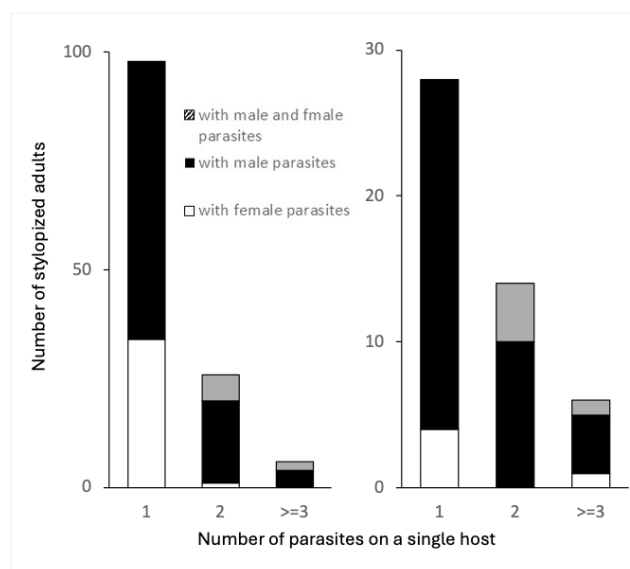


Fig 3. Frequency distributions of the number of *Xenos oxyodontes* parasitic on a single host in *Vespa analis* nests. Left: workers. Right: males. The sex of the parasite on the host is indicated by different patterns shown in the inset on the left graph.

2) Stylozized hornets in bait-trapped samples

Monthly catches, and the percentage of parasitism among bait-collected female and male hornets are shown in Table 1, which compiles results from all trap sites. The annual rate of stylopization ranged from 11.5% to 21.2% in females and from 0% to 13.8% in males. Almost all female hosts carried only female parasites from May to August, while the proportion of hosts with male parasites increased from August onward, with some hosts harboring both female and male parasites (Fig 4). In contrast to the male parasites found in the nests, nearly all (90%) male parasites on the trapped hornets had already emerged, leaving only vacant puparia in their hosts

Female hornets hosting only female parasites were selected to check for first-instar larvae inside the parasites. Among 252 such hosts, 235 (93.3%) had a single female parasite, 16 (6.3%) had two, and one (0.4%) had three female parasites. When multiple female parasites were present on a single host, it was checked whether at least one parasite contained first-instar larvae. Female hosts containing first-instar larvae were found from May through September (Fig 5). While the female hosts collected in May and June were primarily overwintered individuals (queens and workers), nearly all of those collected from August and September were considered current-year workers based on the adult production schedule of *V. analis* (Matsuura and Yamane, 1990). In July, some overwintered females occurred among the current-year workers.

Female parasites found on current-year workers and carrying first-instar larvae are likely preparing to release or in the process of releasing larvae without entering hibernation. Additionally, some female hornets were parasitized by both

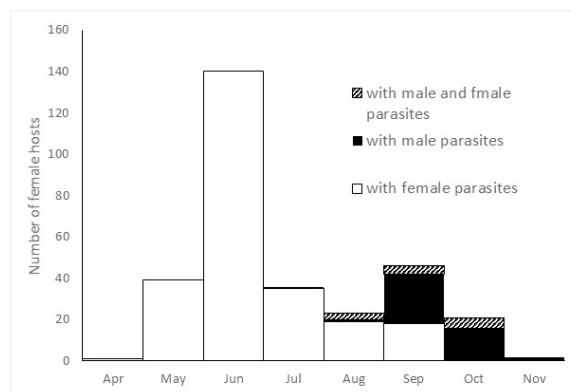


Fig 4. Seasonal changes in the number of stylozized female hornets of *Vespa analis* collected with bait traps. Data are pooled from six study years. Different patterns indicate the sex of the parasite on the host.

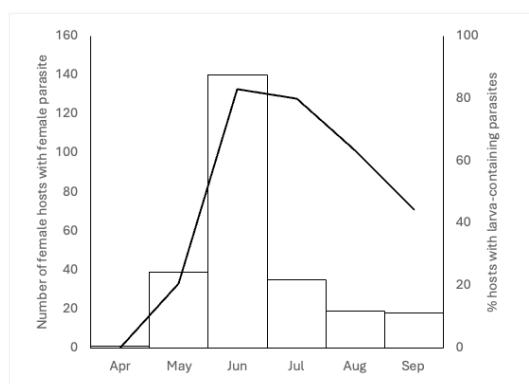


Fig 5. Seasonal changes in the number of female hornets of *Vespa analis* harboring female *Xenos oxydotes* (columns) and the proportion of these hornets whose female parasites contained first-instar larvae (lines).

Table 1. Monthly numbers of females (A) and males (B) of *Vespa analis* collected with bait traps. Pooled catches from five trap sites are given. The percentage of individuals parasitized by *Xenos oxydotes* is shown in parentheses.

A									
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Total
1998	29(0)	73(13.7)	47(40.4)	7(42.9)	9(33.3)	59(13.6)	84(4.8)	3(0)	311(15.1)
1999	1(100)	79(22.8)	90(48.9)	53(35.8)	20(50.0)	214(3.3)	93(4.3)	4(50)	554(19.0)
2000	0	87(9.2)	155(30.3)	30(26.7)	3(66.7)	40(10.0)	9(0)	1(0)	325(21.2)
2001	1(0)	68(4.4)	87(19.5)	13(15.4)	11(27.3)	156(7.7)	46(15.2)	1(0)	383(11.5)
2002	16(6.3)	78(10.3)	66(37.9)	86(23.3)	5(40.0)	51(27.5)	48(10.4)	4(0)	354(21.2)
2003	1(0)	68(8.8)	57(45.6)	3(0)	36(27.8)	134(9.7)	2(100)	0(0)	301(18.9)
Total	48(4.2)	453(11.7)	502(35.5)	192(27.1)	84(35.7)	654(8.9)	282(7.8)	13(15.4)	2228(17.8)

B									
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Total
1998	0	0	0	0	0	3(0)	16(6.3)	0	19(5.3)
1999	0	0	0	0	0	14(7.1)	29(10.3)	0	43(9.3)
2000	0	0	0	0	0	0	6(0)	1(0)	7(0)
2001	0	0	0	0	2(0)	31(9.7)	40(15.0)	0	73(12.3)
2002	0	0	0	2(50.0)	2(0)	5(0)	46(15.2)	3(0)	58(13.8)
2003	0	0	0	0	0	21(4.8)	1(100)	0	22(9.1)
Total	0	0	0	2(50.0)	4(0)	74(6.8)	138(13.0)	4(0)	222(10.8)

female and male parasites (Fig 4). These individuals were clearly from the current year, as no overwintered females have been found with male and female parasites (Makino, 2001; Tatsuta & Makino, 2003). Four of the 12 hosts harboring male and female parasites had at least one female parasite containing first-instar larvae. This suggests that these female parasites, which emerged in the current year, could release first-instar larvae during the remainder of the year.

Discussion

The parasitism rate in *V. analis* nests, defined as the percentage of nests containing at least one stylopized hornet, was 60%, with stylopized hornets accounting for 8.2% of adults across all nests. While slightly higher, these figures are comparable to those reported from another area in central Japan (Nagoya), where 42% of nests and 3.5% of adults were stylopized (Makino et al., 2009). In Wakayama Prefecture, western Japan, Matsuura and Yamane (1990) reported that approximately 27% of *V. analis* nests contained stylopized adults. The actual percentage may have been higher than this since their sample included an unknown number of pre-emergence nests, which inherently lacked stylopized adults. Thus, *X. oxyodontes* appears to be a common parasite in *V. analis* nests across various regions of Japan.

Although the percentage of parasitized nests peaked in September (Fig 1), as reported in Nagoya (Makino et al., 2009), parasitized adults were consistently found in nests from June through October. This finding suggests that first-instar *X. oxyodontes* larvae are transported to the nests by foraging hornets during a long period in the nesting season.

The sex ratio of parasites was male-biased in both workers and males found in the nests (Fig 3). Kifune and Arakawa (1996) also reported a male-biased sex ratio (0.69) in a *V. analis* nest collected in early September. Similarly, Kifune and Maeta (1975) reported a male-biased sex ratio (0.75) in *Xenos vespularum* Kifune & Maeta parasitizing workers and males in the host *Vespa flaviceps* Smith (referred to as "*V. lewisii*" in their paper). The reasons for the male-biased sex ratios in these *Xenos* species remain unclear but may involve factors such as a skewed primary sex ratio or differential mortality between sexes during immature stages.

Among new queens, the two stylopized individuals hosted only female parasites. Although the sample size is too small to draw definitive conclusions, this finding is interesting because a female-biased sex ratio on new queen hosts has also been reported in *X. vespularum* that parasitizes *Vespa flaviceps* (Kifune & Maeta, 1975). Kifune and Maeta (1975) argue that this could be advantageous for the overwintering generation of female parasites because new queens overwinter as opposed to workers. However, in the *Vespa* species, including *V. analis*, workers are known to overwinter when parasitized by female strepsipterans (Matsuura & Yamane, 1990; Tatsuta & Makino, 2003). If *X. oxyodontes* females of the overwintering

generation prefer new queens, there may be a different advantage from *X. vespularum*. Further research should focus on the factors that determine or influence the sex ratio in *Xenos* species.

Mating behavior in *X. oxyodontes* or *X. moutoni* has never been observed under natural conditions. Makino and Yamashita (1998) supposed that *X. moutoni* mates inside the nest because male parasites on bait-collected hornets had almost always emerged. However, their supposition appears incorrect based on the present observation that no male puparia on intranidal hosts had emerged. Then, it is likely that the mating of *X. oxyodontes* occurs outside the nest. Another strepsipteran, *X. vespularum* Rossius, which parasitizes paper wasps (*Polistes* spp.) in Europe, mates after stylopized hosts leave the nest and congregate (Hughes et al., 2004; Beani et al., 2011; Beani et al., 2018). Although such behavior has not been reported in *X. oxyodontes* or *X. moutoni* parasitizing hornets, it is possible that their mating occurs at places where hosts are likely to visit, for example, tree sap holes. Attention should be paid to the extranidal activities of stylopized hornets after they leave their natal nests to uncover the mating behavior of *X. oxyodontes* or *X. moutoni*.

Both sexes of *X. oxyodontes* were consistently found in *V. analis* nests almost throughout the post-emergence season. Even nests collected late in September contained workers infested with male parasites. The development time from egg to adult emergence averages 32 days in *V. analis* (Matsuura & Yamane, 1990), and workers infested with male *X. oxyodontes* spend an average of 7.5 days before leaving their natal nests (Sayama, 2012). Based on these figures, the intranidal hornets with male parasites late in September must have been infected with first-instar parasites in or after early August. These first instars are unlikely to originate from overwintered female parasites because overwintered hosts (queens or workers) are unlikely to survive until early August, as inferred from bait samples. It is more plausible that these first instars were the offspring of first-generation adults, the offspring of overwintered females. Therefore, the parasites on adult hornets late in September are likely to belong to the second generation rather than the first.

The results from the bait traps are consistent with the hypothesis of bivoltinism in *X. oxyodontes*. The bait trap samples indicate that female parasites containing first-instar larvae were present from May through September (Fig 5). The proportion of larvae-containing females was low in May, peaked in June and July, and gradually declined after that. In May and June, the collected hornets were overwintered females (Makino, 2001; Tatsuta & Makino, 2003; Kudô et al., 2024b), meaning the female parasites on them were also from the overwintering generation. However, workers produced that year began to appear in July, and females collected after August were current-year workers (Makino, 2001). Female parasites on these current-year workers are likely the offspring of overwintering females, and it is reasonable to assume that

the first-instar larvae released from these females on current-year workers are brought back to the nests to invade larvae and produce the next generation of adults.

Overwintered stylopized hornets always host only female parasites. No cases have been reported where overwintering females in hibernacula have male parasites, nor have overwintered females trapped early in the season been found with male parasites. The explanation for this outcome is that, most likely, male parasites emerge before overwintering, leaving a hole between tergites, which would make the host short-lived and unlikely to survive until or through overwintering (Maeta, 1963b; Beani et al., 2011). Therefore, wasps parasitized by male and female parasites are reasonably assumed to have been parasitized in the current year. If the female parasite on these hosts contains first-instar larvae, these larvae would likely develop into adults of the second generation.

This study indicates that *X. oxyodontes* parasitizing *V. analis* has two generations per year in Ibaraki Prefecture, central Japan. Kudô et al. (2024a) also suggest multivoltinism in *X. oxyodontes* in Niigata, which is relatively close to the present study area in Ibaraki. Bivoltinism is also known in *X. vesparum* parasitizing *Polistes dominulus* (Christ) in Europe (Beani et al., 2011; Beani et al., 2018).

The voltinism of the parasite may vary with its geographical distribution. Sayama (2012) proposed univoltinism for *X. oxyodontes* in Hokkaido, northern Japan, based on seasonal changes in stylopized hosts of *V. analis* collected with bait traps and visits by stylopized females to hibernation sites in summer. This proposition is reasonable, given the shorter active period of *V. analis* in Hokkaido compared to warmer regions, including the present study sites. In contrast, Matsuura and Yamane (1990) reported that first-instar larvae of *Xenos moutoni* consistently occur from June through November, and Matsuura (1995) asserted multivoltinism (at least three generations per year) in southern Japan. Since *X. moutoni* has a wide range of hosts (Nakase & Kato, 2013), including *V. mandarinia* and *V. simillima*, which have much longer nesting periods than *V. analis* (Matsuura & Yamane, 1990), the parasite may have more than two generations depending on the host, particularly in warmer regions of Japan.

Acknowledgments

We thank K. Kudô for his valuable comments and suggestions on the manuscript. I am also grateful to two anonymous referees for improving the manuscript. The following persons and organizations were helpful in trap-sampling or nest collection: K. Kimura, K. Sayama, Ushiku Nature Reserve (Ushiku City), Takasaki Nature Park (Tsukuba City), and Kukizaki Fire Station (Tsukuba City). This work was partly conducted under the research scheme of the Forestry and Forest Products Research Institute “Assessment of elements of natural environments affecting human amenity and health (FY2001-2005).”

References

- Beani, L., Dallai, R., Mercati, D., Cappa, F., Giusti, F. & Manfredini, F. (2011). When a parasite breaks all the rules of a colony: morphology and fate of wasps infected by a strepsipteran endoparasite. *Animal Behaviour*, 82: 1305-1312. <https://doi.org/10.1016/j.anbehav.2011.09.012>
- Beani, L., Marchini, D., Cappa, F., Petrocelli, I., Gottardo, M., Manfredini, F., Giusti, F., Dallai, R. (2017). Subtle effect of *Xenos vesparum* (Xenidae, Strepsiptera) on the reproductive apparatus of its male host: Parasite or parasitoid? *Journal of Insect Physiology*, 101: 22-30. <https://doi.org/10.1016/j.jinsphys.2017.06.010>
- Beani, L., Cappa, F., Manfredini, F. & Zaccaroni, M. (2018). Preference of *Polistes dominula* wasps for trumpet creepers when infected by *Xenos vesparum*: A novel example of co-evolved traits between host and parasite. *PLoS ONE*, 13: e0205201. <https://doi.org/10.1371/journal.pone.0205201>
- Benda, D., Pohl, H., Nakase, Y., Beutel, R. & Straka, J. (2022). A generic classification of Xenidae (Strepsiptera) based on the morphology of the female cephalothorax and male cephalotheca with a preliminary checklist of species. *ZooKeys*, 1093: 1-134. <https://doi.org/10.3897/zookeys.1093.72339>
- Brock, R.E., Cini, A. & Summer, S. (2021). Ecosystem services provided by aculeate wasps. *Biological Reviews*, 96: 1645-1675. <https://doi.org/10.1111/brv.12719>
- Cappa, F., Manfredini, F., Dallai, R., Gottardo, M. & Beani, L. (2014). Parasitic castration by *Xenos vesparum* depends on host gender. *Parasitology*, 141: 1080-1087. <https://doi.org/10.1017/S003118201400047X>
- Edwards, R. (1980). *Social Wasps. Their Biology and Control*: Rentokil: East Grinstead, 398 p.
- Hughes, D.P., Beani, L., Turillazzi, S. & Kathirithamby, J. (2003). Prevalence of the parasite Strepsiptera in *Polistes* as detected by dissection of immatures. *Insectes Sociaux*, 50: 62-68. <https://doi.org/10.1007/s000400300010>
- Hughes, D.P., Kathirithamby, J., Turillazzi, S. & Beani, L. (2004). Social wasps desert the colony and aggregate outside if parasitized: Parasite manipulation? *Behavioral Ecology*, 15: 1037-1043. <https://doi.org/10.1093/beheco/arl111>
- Japan Meteorological Agency (2024). Historical weather data retrieval. <https://www.data.jma.go.jp/stats/etrn/index.php>.
- Kanzaki, N., Makino, S., Kosaka, H., Sayama, K., Hamaguchi, K. & Narayama, S. (2023). Nematode and strepsipteran parasitism in bait-trapped and hand-collected hornets (Hymenoptera, Vespidae, *Vespa*). *Insects*, 14: 398. <https://doi.org/10.3390/insects14040398>
- Kathirithamby, J. (2009). Host-parasitoid associations in Strepsiptera. *Annual Review of Entomology*, 54: 227-249. <https://doi.org/10.1146/annurev.ento.54.110807.090525>

- Kifune, T. & Maeta, Y. (1975). A new subgenus and new species of the genus *Xenos* (Strepsiptera, Stylopidae) from Japan (Studies on the Japanese Strepsiptera III). *Kontyû*, Tokyo, 43: 446-455.
- Kifune, T. & Arakawa, R. (1996). Records of three strepsipterans from Kochi Prefecture, Shikoku, Japan. *Gensei*, 68: 13-15. (In Japanese.)
- Kudô, K., Oyaizu, W., Kusama, R., Yamagishi, K., Yamaguchi, Y. & Koji, S. (2024a). Effect of environmental factors on strepsipteran parasite abundance in vespine wasps (Hymenoptera: Vespidae). *Sociobiology*, 71: e10347. <https://doi.org/10.13102/sociobiology.v71i1.10347>
- Kudô, K., Oyaizu, W., Kusama, R., Yamagishi, K., Yamaguchi, Y. & Koji, S. (2024b). Mating and post-hibernation ovarian development in stylopized and non-stylopized queens of the hornet *Vespa analis* (Hymenoptera Vespidae). *Ethology, Ecology and Evolution*, 35: 86-95. <https://doi.org/10.1080/03949370.2023.2213194>
- Maeta, Y. (1963a). Some biological notes on *Pseudoxenos iwatai* Esaki (I) Its biology and life history. *Kontyû*, Tokyo, 31: 1-14. (In Japanese with English summary.)
- Maeta, Y. (1963b). Some biological notes on *Pseudoxenos iwatai* Esaki (II) Its biology and life history. *Kontyû*, Tokyo, 31: 113-126. (In Japanese with English summary.)
- Maeta, Y. & Kifune, T. (1990) Strepsipterous insects and their lives (5). *Insectarium*, 27: 198-205. (In Japanese.)
- Makino, S. (2001). Seasonal changes in levels of parasitism and sex ratio of *Xenos moutoni* in the Japanese hornet, *Vespa analis insularis* Dalla Torre (Hymenoptera, Vespidae), collected with attractant traps. *Tijdschrift voor Entomologie*, 144: 217-222. <https://doi.org/10.1163/22119434-900000087>
- Makino, S. & Sayama, K. (2005). Species compositions of vespine wasps collected with bait traps in recreation forests in northern and central Japan (Insecta, Hymenoptera, Vespidae). *Bulletin of Forestry and Forest Products Research Institute*, 4: 283-289.
- Makino, S. & Yamashita, Y. (1998). Levels of parasitism by *Xenos moutoni* du Buysson (Strepsiptera, Stylopidae) and their seasonal changes in hornets (Hymenoptera: Vespidae, *Vespa*) caught with bait traps. *Entomological Science*, 1: 537-543.
- Makino, S., Yamaura, Y. & Yamauchi, H. (2009). Smaller nests of the hornet *Vespa analis* (Hymenoptera, Vespidae) are more severely affected by the strepsipteran parasite *Xenos moutoni* (Strepsiptera, Stylopidae) than are larger nests. *Insectes Sociaux*, 57: 83-90. <https://doi.org/10.1007/s00040-009-0057-6>
- Matsuura, M. (1995). *Social Wasps of Japan in Color*: Hokkaido University Press, 353 p.
- Matsuura, M. & Yamane, S. (1990). *Biology of the Vespine Wasps*: Springer, 323 p. <https://doi.org/10.1007/978-3-642-75230-8>
- Nakase, Y. & Kato, M. (2013). Cryptic diversity and host specificity in giant *Xenos* strepsipterans parasitic in large *Vespa* hornets. *Zoological Science*, 30: 331-336. <https://doi.org/10.2108/zsj.30.331>
- Otaki, N. (2005). [Allergic symptoms to stings of wasps and bees.] In Matsuura M. et al. (eds.) [“Preventions and Treatments of Stings by Wasps and Bees. 2nd ed.”] Forestry and Timber Manufacturing Safety & Health Association, Tokyo, 211-257. (In Japanese)
- Oyaizu, W. & Kudô, K. (2013). Seasonal changes in the number of vespine wasps and levels of parasitism by *Xenos moutoni* (Strepsiptera, Stylopidae) collected with attractant traps in Matsunoyama forest, Tokamachi city, Japan. *Bulletin of the Faculty of Education. Natural Sciences, Niigata University*, 6: 49-57.
- Perkins, J.B & Yates, A.B. (2017). Pathologic findings in Hymenoptera sting fatalities. In T. M. Freeman & J. M. Tracy (Eds.), *Stinging insect allergy* (pp. 101-108). Springer. https://doi.org/10.1007/978-3-319-46192-2_6
- Sayama, K. (2012). Host utilization and seasonal occurrence of the hornet parasite *Xenos moutoni* in Hokkaido, northern Japan. *Insects and Nature*, 47(3): 21-24. (In Japanese)
- Schmid-Hempel, P. (1998). *Parasites in Social Insects*: Princeton University Press, 409 p. <https://doi.org/10.1515/9780691206851>
- Tatsuta, H. & Makino, S. (2003). Rate of strepsipteran parasitization among overwintered females of the hornet *Vespa analis* (Hymenoptera: Vespidae). *Environmental Entomology*, 32: 175-179. <https://doi.org/10.1603/0046-225X-32.1.175>

