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Effect of Environmental Factors on Strepsipteran Parasite Abundance in Vespine Wasps (Hymenoptera: Vespidae)

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Abstract

Xenos is a strepsipteran genus parasitizing hornets of the *Vespa* spp. In Japan, parasitism levels in hornets have been examined for two *Xenos* species. These studies have indicated that parasitism levels vary with the seasons but are inconsequential compared to other potential factors that reduce the number of host colonies. However, a comprehensive analysis of parasitism levels accounting for various factors such as host species, seasons, and years was not conducted. To address this gap, we conducted a four-year study in which we collected *Vespa* spp. using bait traps and assessed parasitism levels concerning those factors. Additionally, we evaluated the number of male and female *Xenos* parasites throughout the seasons. A total of 11,766 wasps from six hornet species were collected, with three of the species conspicuously parasitized by two *Xenos* parasites: *Vespa analis* (Fabricius, 1775) parasitized by *Xenos oxyodontes* (Nakase & Kato, 2013) and *Vespa mandarinia* (Smith, 1852) and *Vespa ducalis* (Smith, 1852) parasitized by *Xenos moutoni* (Buysson, 1903). Levels of parasitism by *X. oxyodontes* exceeded those by *X. moutoni*. Seasonal and yearly variations influenced the levels of parasitism by the two *Xenos* spp. We observed that male and female *Xenos* parasites emerged simultaneously over 2 – 3 months, suggesting the likelihood of multivoltinism in temperate regions. Our study also examined several key factors influencing the fluctuations in the population size of these parasites.

Introduction

Vespa is a genus within the subfamily Vespinae and is the largest and most representative group of social wasps (Matsuura, 1991). These wasps play a significant ecological role as predators of arthropods, exerting a potential influence on arthropod communities in various habitats worldwide through their hunting behavior. Additionally, these wasps frequently visit flowers, contributing to pollination efforts like bees.

Numerous natural enemies exist, preying on adult wasps both inside and outside their nests, targeting both immature and adult stages (see review by Matuura & Yamane, 1990). Beyond these known natural threats, adult wasps contend with endoparasites such as Stylopidae, Conopidae, and Nematoda. *Xenos* is a strepsipteran genus whose members parasitize *Vespa* wasps (Kinzelbach, 1971). Adult wasps engage in activities such as visiting, consuming tree sap for sustenance, and nourishing their larvae. In contrast, the first instar larvae of the *Xenos* parasite (hereafter referred to as triungulin larvae) await an opportunity to attach themselves to host nests. In Japan, *Xenos* spp. parasitize six out of seven *Vespa* spp. across all the main islands (Kifune, 1992; Kifune & Makino, 1996). Some aspects of the behavioral features of parasitized adult *Vespa* have been investigated. For example, parasitized workers cease the absence of foraging activities, instead opting to remain within their nests for rest (Matsuura & Yamane, 1990). Consequently, increased non-participation

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in foraging activities has limited *Vespa crabro* (Linnaeus, 1758) colony development (Matsuura & Yamane, 1990). The effects of the parasitism are also discernible in males and queens, resulting in a loss of reproductive capabilities.

The levels of parasitism in *Vespa* spp. have been extensively investigated across various regions in Japan (Makino & Yamashita, 1998; Makino, 2001; Tatsuta & Makino, 2003; Oyaizu & Kudô, 2013; Kanzaki et al., 2023; Kudô et al., 2024). These studies have revealed common trends among *Vespa* spp.: (1) *Vespa analis* exhibits the highest levels of parasitism; (2) while most host hornets are singly parasitized, there are instances of multiple parasitizations in certain individuals; (3) the male parasites emerge late in the season. Our understanding of the interaction between the parasites and host hornets has been gradually expanded. However, most of these studies were conducted when only known *Xenos* species existed. Nakase & Kato (2013) conducted a molecular and morphological phylogenetic analysis of *Xenos* specimens collected from East Asian hornets, showing two distinct *Xenos* spp., *Xenos moutoni,* and *Xenos oxyodontes*. Furthermore, they demonstrated that these two species exhibit differences in their host utilization patterns: *X. oxyodontes* targets *V. analis* and *Vespa simillima* (Smith, 1868) primarily, whereas *X. moutoni* is associated with other species in *Vespa* spp. The implication of this phylogenetic analysis underscores the need for an independent reevaluation of parasitism levels and host utilization patterns by these *Xenos* spp.

Kanzaki et al. (2023) conducted a study after the publication of phylogenetic analyses by Nakase & Kato (2013). Kanzaki et al. (2023) compared the level of parasitism between trapped hosts and hand-collected specimens. The results indicated a higher level of parasitism in the former group than in the latter, suggesting that these parasites may manipulate their host to visit feeding sites. In addition, Kanzaki et al. (2023) provided support for the previously identified trends in (1), (2), and (3) analyzing their data on parasitism by the two *Xenos* spp. separately. However, parasitism levels have not been analyzed yet, considering various factors, such as host species, seasons, and years. In particular, the influence of sampling years on parasitism levels remains a possibility that has not been adequately explored (Makino, 2001).

In this study, we examined parasitism by two *Xenos* spp. in *Vespa* spp., which were collected using bait traps over four years in Niigata, central Japan. Our previous study elucidated six *Vespa* spp. with bait traps spanning from May to November in the study locality (Kudô et al., 2021). Among these species, *V. analis*, *Vespa ducalis* and *Vespa mandarinia* predominated, allowing us to analyze parasitism levels across different host species, seasons, and years.

Materials and Methods

We monitored hornets in two distinct locations, precisely, Sakata Park (hereafter referred to as SP) (37º 49' N, 138º 52 'E) and the Niigata University Campus (hereafter referred to as CNU) (37º 52' N, 138º 56' E), situated in Niigata city, central Japan, during the period spanning from 2009 to 2012. An approximate distance of 8 km geographically separated these locations. SP primarily featured *Salix babylonica* (Linnaeus var. Babylonica, 1753) and *Celtis sinensis* (Nakai, 1914) as the dominant tree species in SP, while near the CNU, Japanese black pines (*Pinus thunbergii*, Parl, 1868) were strategically planted to serve as a windbreak. Hornets were collected using bait traps consisting of a mixture of 100 mL of water and an equal quantity of grape juice. For the bait containers, we utilized clear plastic bottles with a capacity of 2,000 mL, each equipped with a small square opening measuring 3 × 3 cm in the upper section of the bottle.

Hornets that entered the traps became entrapped within the containers and subsequently perished. These traps were affixed to trees at a height of 1.5 m above the ground and were subjected to weekly inspection from May to November annually. The bait was replenished every week, and the trapped wasps were preserved in a solution of 70% ethanol. As for the SP and CNU locations, we deployed 30 and 22 traps, respectively, positioned at 40–50 m intervals, primarily along the established walking trails.

Xenos parasites were observed inhabiting the terga of their host wasps. Regardless of the host species or gender, female parasites consistently occupied the space between the fifth and sixth terga of the hosts. At the same time, males were primarily located between the fourth and fifth terga, with occasional occurrences between the third and fourth terga (Makino and Yamashita, 1998). Upon encountering *Xenos* parasites their position was recorded to determine their gender. Subsequently, the count of parasites within the host wasp was determined.

We analyzed the effect of year, month, and their interaction on the parasitism rates in *V. analis* (the proportion of the hornet individuals attacked by *X. oxyodontes*) using a generalized linear model with a binomial error distribution. We explored the same model for evaluating parasitism by *X. moutoni* while considering year, month, host species (*V. ducalis* or *V. mandarinia*), and the interaction between year and month as the contributing factors. Statistical significance was tested using a likelihood ratio test. We did not include parasitism data for *V. crabro*, *Vespa dybowskii* (André, 1884), and *V. simillima* in the analysis due to the limited number of captures (see Results). Fisher's exact tests analyzed differences in the parasitism rate between two *Xenos* species. Differences in the total number of *Xenos* parasite individuals and their parasitism rate between host hornet species were tested using Wilcoxon singed-rank tests. The analysis used R version 4.3.1 (R Core Team, 2023)

Results

We examined a total of 11,766 wasps representing six hornet species. No individuals of *V. crabro* and *V. dybowskii* were found to be parasitized by *Xenos* spp. In contrast, a small number of *V. simillima* individuals (N = 5) were parasitized (Table 1). Due to the low number of parasitized individuals, this species was excluded from subsequent analyses. Conversely, 7.38% of *V. analis* individuals were parasitized by *X. oxyodontes*, while 3.11% and 0.85% of *V. mandarinia* and *V. ducalis* individuals, respectively, were parasitized by *X. moutoni* (Table 1). The parasitism rate of *X. oxyodontes* was significantly higher than that of *X. moutoni* (Fisher's exact tests, $p < 0.0001$).

Due to the significant variations in parasitism rates observed between the two *Xenos* spp., we independently analyzed the factors influencing parasitism in each species. The number of *X. oxyodontes* exhibited monthly fluctuations within *V. analis* (Fig 1A), with a minor peak occurring in June and a gradual increase later in the season. Given the differing

Table 1. Number of *Vespa* spp. and *Xenos* parasites examined.

Host species	Number of hornets examined	Number of *Xenos* parasites (%)
Vespa analis	4758	339 (7.12)
V. mandarinia	1377	41 (2.98)
V. ducalis	4268	36 (0.84)
V. simillima	550	5 (0.91)
V. crabro	394	0 (0)
V. dybowskii	99	0 (0)
Toal	11446	421 (3.68)

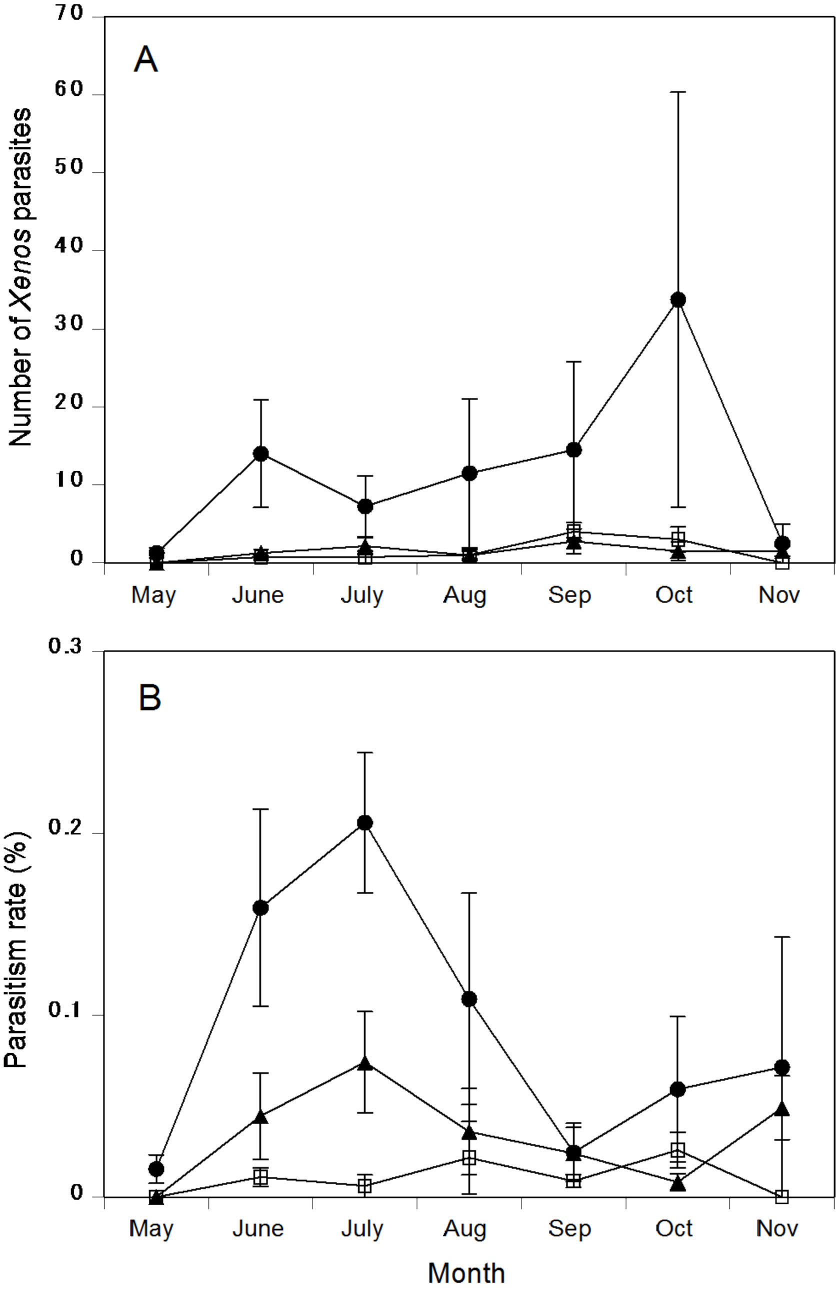

Fig 1. (A) Seasonal variation in the monthly count of *Xenos* parasites (mean ± SE) and (B) the parasitism rate (the number of parasitized hornets divided by the total number of captured hornets). Solid circles, solid triangles, and open squares represent *V. analis*, *V. mandarinia*, and *V. ducalis*, respectively.

quantities of host hornets collected over the four years, we computed the parasitism rate (i.e., the number of parasitized hornets divided by the total number of captured hornets) each month across these four years (Fig 1B). This analysis revealed significant variations in the parasitism rate across different months ($\chi = 165.08$, df = 6, $p < 0.001$). A notable peak was observed during June and July, with a subsequent decline in later months. Furthermore, the parasitism rate displayed year-to-year differences ($\chi = 196.52$, df = 3, $p < 0.001$), declining from 0.13 in 2009 to 0.01 in 2012. A significant interaction effect was detected between year and month ($\chi = 58.45$, df = 18, $p < 0.001$).

The number of *X. moutoni* exhibited variation across months in both *V. mandarinia* and *V. ducalis*, albeit without a remarkable peak, in contrast to *X. oxyodontes* (Fig 1A). Monthly fluctuations in the parasitism rate in *V. mandarinia* resembled those observed in *X. oxyodontes* within *V. analis* (Fig 1B). In contrast, the parasitism rate in *V. ducalis* remained relatively stable throughout the months. No significant difference was observed in the number of *X. moutoni* between *V. mandarinia* and *V. ducalis* (Wilcoxon signed-rank test, $z = 0.509$, $p = 0.611$). However, a significant difference in the parasitism rate was observed between these host hornet species (Wilcoxon signed-rank tests, $z = 2.173$, $p = 0.030$). The generalized linear model demonstrated that the parasitism rate by *X. mouton*i exhibited monthly variation ($\chi = 13.82$, df = 6, $p = 0.032$) with a higher rate recorded in *V. mandarinia* compared to *V. ducalis* ($\chi = 9.12$, df = 1, $p < 0.001$). Additionally, the parasitism rate displayed variations across different years ($\chi = 21.77$, df = 3, $p < 0.001$).

The sex ratio of the parasites exhibited significant seasonal variations (Fig 2). In May and June, only female *X. oxyodontes* were identified. Male individuals were initially documented in late July, with their population subsequently growing. A similar delay in male emergence was observed in *X. moutoni*.

The number of parasites per host ranged from one to seven in *X. oxyodontes*, with approximately 70% of hosts exhibiting a single parasite (Table 2). On the contrary, approximately 95% of hosts exhibited a single parasite, i.e., *X. moutoni*. The fraction of a single parasite was significantly greater in *X. moutoni* than in *X. oxyodontes* (Fisher's exact tests, $p < 0.0001$).

Table 2. Number of *Xenos* parasites in a single host.

Host species	Number of *Xenos* parasites						
	1	2	3	4	5	6	7
V. analis	234	70	12	14	4	3	1
V. mandarinia	36	0	1	0	0	0	0
V. ducalis	33	2	0	0	0	0	0

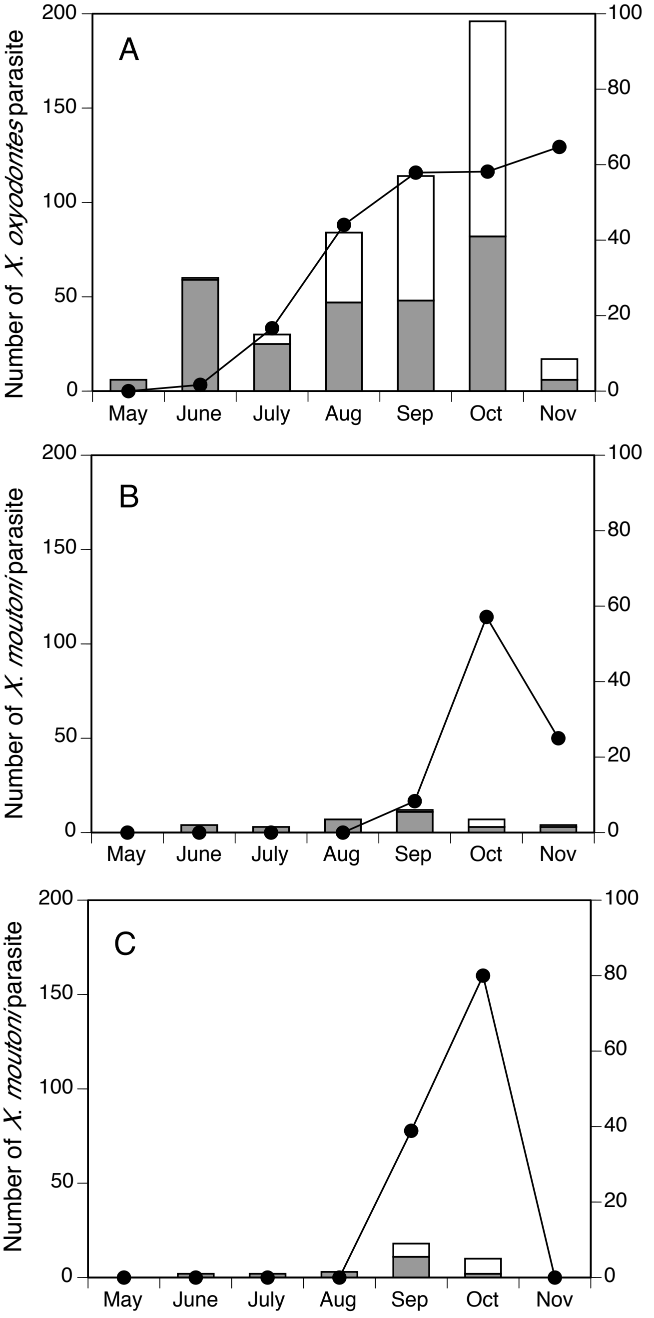

Fig 2. Seasonal variation in the monthly count of male and female *Xenos* parasites and the male ratio in them. Pooled data from *V. analis* (A), *V. mandarinia* (B), and *V. ducalis* (C).

Discussion

In this study, we examined the levels of parasitism by two sympatric *Xenos* spp. in three Japanese hornets. We also compared male emergence timing and the number of parasites per host among these parasite species. *X. oxyodontes* exhibited a higher level of parasitism compared to *X. moutoni*. While the timing of male emergence was nearly identical between the two parasite species, we observed a greater incidence of multiple parasitism in *X. oxyodontes.*

This study revealed a higher level of parasitism in *V. analis* compared to *V. mandarinia* and *V. ducalis*. This finding aligns with previous studies (Makino & Yamashita, 1998; Makino, 2001). However, Oyaizu & Kudô (2013) reported a higher level of parasitism in *V. mandarinia* compared to *V. analis*. Kanzaki et al. (2023) reported that the affinity between *V. analis* and *X. oxyodontes* may be greater than that observed between other *Vespa* spp. and *X. moutoni*. Another plausible explanation could be differences in female fecundity between the two *Xenos* spp. Female host hornets frequently visit tree saps to release triangulin larvae, which are parasitic on the host hornets' larvae. Triangulin larvae infiltrate host nests by attaching themselves to other adult hornets. If the number of triangulin larvae produced by *X. oxyodontes* exceeds that of *X. moutoni*, it is likely to result in varying levels of parasitism between the two *Xenos* spp. However, comparative studies on the fecundity of female parasites are scarce, with limited information available from a single study on a parasitized worker of *V. mandarinia* (Matsuura, 1975). Matsuura (1975) reared a parasitized worker in the laboratory and showed that the number of triangulin larvae observed to emerge from an adult female *X. moutoni* was 36,739 individuals.

Variations in the parasitism rates were observed across different months for both *Xenos* spp. Specifically, the highest rates were observed in June and July (see also *X. oxyodontes* in Makino, 2001). Parasitized workers and queens emerge after hibernation during these months (Makino & Yamashita, 1998; Tatsuta & Makino, 2003; Kudô et al., 2024). This phenomenon leads to elevated levels of parasitism.

Multiple parasitism was more frequently observed in *X. oxyodontes* than in *X. moutoni*. However, the rates of multiple parasitism did not exhibit consistency across previous studies. For example, Makino & Yamashita (1998) reported a similar rate of multiple parasitism between the parasite species, with 22% in *X. oxyodontes* compared to 19% in *X. moutoni*. Conversely, a recent study by Kanzaki et al. (2023) aligns closely with our current findings. Kanzaki et al. (2023) suggested that the population density and behavior of host wasps may influence the variance in multiple parasitism.

Additionally, it appears likely that differences in the fecundity of females in the two *Xenos* spp. contribute to the occurrence of single or multiple parasitisms. A higher number of triangulin larvae from *X. oxyodontes* infiltrating *V. analis* nests corresponds to a greater number of parasite larvae invading host larvae within the nests.

In *X. oxyodontes*, male emergence commences in late July, and the number of individuals is notably comparable to that of females from August to October, suggesting that mating activities were continuous during this period. If the female parasites within host workers mate in August, these workers overwinter and, similar to their parasitized queens, commence visits to tree sap in the following spring (Matsuura & Yamane, 1990; Makino & Yamashita, 1998; Tatsuta & Makino, 2003). Indeed, parasitized *V. analis* individuals with smaller body sizes overwinter and can be captured using bait traps (Makino, 2001; Kudô et al., 2024). If mating among *X. oxyodontes* occurs continuously between August and October, the possibility of multivoltinism in this species becomes plausible. Makino (2001) noted that the voltinism of this parasite species remains unclear but is feasible if the estimated developmental duration of immature parasites is accurate. In contrast to *X. oxyodontes*, the emergence of *X. moutoni* males was exclusively observed in September and October, suggesting that mating is restricted to these two months. However, additional information will be required in future studies owing to the small sample size of *X. moutoni* in this study.

This study is the first to demonstrate notable variation in *Xenos* parasitism levels within *Vespa* spp. across different years. Previous research has established the variability of parasitism levels among various regions or nests (Matsuura & Yamane, 1990). Furthermore, Makino (2001) conducted a three-year study utilizing bait traps to collect parasitized individuals of *V. analis*, revealing distinct patterns in the parasitism rates observed across those years. Both the current study and previous investigations suggest the existence of several key factors influencing the fluctuations in parasite population size. Factors related to host hornets, such as their behaviors and the success of hibernation in parasitized individuals, can potentially impact parasitism levels.

Authors' Contributions

KK: conceptualization, methodology, investigation, writing (original draft, review & editing);
WO: investigation, writing-original draft, resources;
RK: investigation, writing-original draft;
KY: investigation, writing-original draft;
YY: investigation, writing-original draft;
SK: methodology, writing (original draft, review & editing).

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