

Social wasps desert the colony and aggregate outside if parasitized: parasite manipulation?

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Infection of the paper wasp, *Polistes dominulus* (Christ), by the strepsipteran parasite *Xenos vesparum* Rossi results in a dramatic behavioral change, which culminates in colony desertion and the formation of extranidal aggregations, in which up to 98% of occupants are parasitized females. Aggregations formed on prominent vegetation, traditional lek-sites of *Polistes* males, and on buildings, which were later adopted as hibernating sites by future queens. First discovered by W.D. Hamilton, these aberrant aggregations are an overlooked phenomenon of the behavioral ecology of this intensively studied wasp. For 3 months in the summer of 2000, during the peak of colony development, we sampled 91 extranidal aggregations from seven areas, numbering 1322 wasps. These wasps were parasitized by both sexes of *X. vesparum*, but males were more frequent from July until mid-August, during the mating season of the parasite. Aggregations were present for days at the same sites (in one case a leaf was occupied for 36 consecutive days) and were characterized by extreme inactivity. After artificial infection, parasitized “workers” deserted the nest 1 week after emergence from their cell and before the extrusion of the parasite through the host cuticle. Infected individuals did not work, were more inactive, and did not receive more aggression than did controls. We suggest that early nest desertion and subsequent aggregations by parasitized nominal workers and “future queens” is adaptive manipulation of host behavior by the parasite to promote the completion of its life cycle. *Key words*: aggregation, behavioral manipulation, nest desertion, *Polistes*, Strepsiptera. [*Behav Ecol* 15:1037–1043 (2004)]

Primitively eusocial wasps of the genus *Polistes* have played a pivotal role as “hypothesis generating model organisms” (Turillazzi and West-Eberhard, 1996) in sociobiology (Pardi, 1948) and kin selection theories (Hamilton, 1964). Their annual, unenveloped, small nests, founded by one or more overwintered females, are relatively easy to study. This, in addition to their widespread occurrence in temperate zones, has resulted in a voluminous literature regarding their behavior (Reeve, 1991). One species in particular, *Polistes dominulus* (Christ), has been claimed to the “most well studied social wasp” (Queller et al., 2000). Therefore, the discovery by W.D. Hamilton of previously unknown extranidal aggregations of *P. dominulus* consisting of females parasitized by the strepsipteran *Xenos vesparum* (dissected by J. Kathirithamby) at a time when colonies were fully active (August 1998, Tuscany, Italy, see Hughes, 2002), was novel and unexpected. Why should infected individuals abandon their nest and aggregate outside when the colony is a “fortress” for both parasites and wasps (Schmid-Hempel, 1998)? Extranidal aggregations, which consist of healthy future queens, are common only at the end of the reproductive phase (Pardi, 1942; West-Eberhard, 1969) and during diapause (Hunt et al., 1999; Turillazzi, 1980). Hamilton’s aberrant, early aggregations of females could be an example of parasite-induced change in host behavior: “After all, if they can so completely castrate the wasps without killing them, an ability obviously evolved in an association through an immense period of time, it will not be surprising to find that they also manipulate the behavior” (Hamilton WD, e-mail to Laura Beani, 3-09-1998, 11:35 hrs).

The order Strepsiptera are obligate endoparasitic insects that are known to parasitize seven insect orders, including solitary and social Hymenoptera (Kathirithamby, 1989). They are “parasitic castrators” (Baudoin, 1975), because they induce sterility in their hosts (Strambi and Strambi, 1973). These macroparasites exhibit extreme sexual dimorphism (Figure 1a,b): the short-lived (usually less than 5 h) winged adult males and the first instar larvae are the only free-living stages, whereas the neotenic larviform adult females are permanently parasitic. Infection by *X. vesparum* (which is termed stylopization after the family Stylopidae that infects wasps and bees) begins with the entry of the first instar larva into a *P. dominulus* larvae (all host larval stages are susceptible). After successive endoparasitic stages and host pupation, the last instar *X. vesparum* extrudes its anterior region through the intersegmental membranes of the adult host. The male forms a cephalotheca and pupates, and the female forms a cephalothorax and becomes a neotenic nonpupating adult. Adult males emerge from their puparia and fly off to inseminate a female through the brood canal opening in her cephalothorax. Females are viviparous and embryonic development occurs within the hemocoel (Kathirithamby, 2000), and the first instar larvae emerge via the brood canal opening. Emergence occurs after a stylopized wasp (containing a gravid female *X. vesparum*) alights on flowers, and subsequent transport of first instars to the nest is phoretic, via a foraging wasp. However, stylopized wasps might land on nests and first instars emerge here to find new hosts (Hughes et al., 2003). Only adult female *X. vesparum* overwinter (with their host); males die soon after fertilizing the females.

In Tuscany, Italy, fertilized *P. dominulus* emerge from diapause in March and begin nest construction, either singly (haplometrosis) or as a group (pleometrosis), with the first workers emerging in May/June and the first sexuals emerging in July/August (Pardi, 1942; Reeve, 1991). Colony decline occurs in September. Nests are very common on buildings, for

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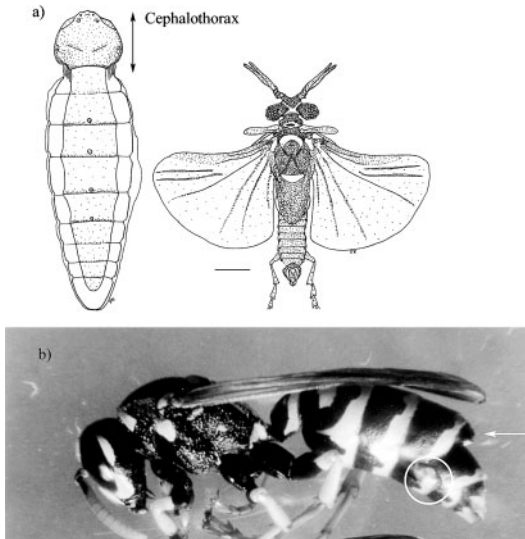


Figure 1
 (a) A line diagram of an adult female and adult male *X. vesparum*. The double-pointed arrow details the female cephalothorax, which extrudes through the host. The scale bar = 0.8 mm (original drawing by Jeyaraney Kathirithamby, copyrighted, see www.tolweb.org/tree/). (b) A stylized female *P. dominulus*. The arrow shows the more evident cephalotheca of the *X. vesparum* male puparium, and the more cryptic female cephalothorax is circled.

example, under roof tiles and eaves. Mating occurs at lek-sites in August/September (large trees and poles around houses where nests are constructed; Beani, 1996), and females overwinter in crevices of buildings and under roof tiles. Parasitism of *P. dominulus* by *X. vesparum* was common in Tuscany: 58% of nests were infected; in these nests 28% of brood, but none of adults, were stylized (Hughes et al., 2003).

The "intimate interaction" (Combes, 2001) between host and parasite is particularly long in *Xenos/Polistes* systems as infection begins at the immature host stage (larval wasp) and proceeds into adulthood. Female *Xenos* remain associated with their hosts throughout adulthood and, where the female overwinters, the association can last up to 1 year. Nevertheless, previous behavioral records are scant and limited to lethargy by infected workers (Fitzgerald, 1938; Hubbard, 1892), communal overwintering by healthy and stylized females (Turillazzi, 1980), and the nonparticipation of infected "queens" in colony life (Pardi, 1942). However, two reviews of colony symbionts do not mention them (Nelson, 1968; Yamane, 1996). In the present study, we document the occurrence of extranidal aggregations of wasps at seven sites in Tuscany, including Hamilton's first record area, throughout the season. We also document the behavior of individuals within these aggregations through focal animal observations and mark-recapture techniques. Importantly, we perform controlled infections of workers under laboratory conditions to determine when they leave the nest and if desertion is preceded by aggression from nest mates, or if stylized individuals contribute to own indirect fitness through work performance.

METHODS

Data collection in the field: single stylized wasps, aggregations, and nests

We checked seven field sites within 15 km of Florence, Italy (43°45' N, 11°18' E), from 8 June–23 August 2000 for the presence of extranidal wasps. For one site (area 1, close to

Florence airport) we sampled aggregations until 23 October, in order to document the changes in aggregations over the season. First, we collected single stylized wasps on vegetation or in flight ($n = 21$). After the 16 July, we sampled "aggregations," here defined as four or more wasps in contact, from vegetation surrounding buildings with a high density of nests. We placed an insect sweep net over each aggregation, and because of the extreme inactivity of the gathered wasps, escapes were uncommon. Sampling of aggregations was divided by week, with 16–23 July being week 1 and 7–15 October being week 12. We noted the location, size, and parasite prevalence (the proportion of infected individuals) of each aggregation. Collections occurred from 0800–2000 h, plus seven nightly checks (0200–0700 h). Wasps that were evidently parasitized were checked for sex of both wasps and parasites and released, whereas a few ($n = 27$) that did not appear evidently parasitized, that is, with extruded cephalothorax and/or cephalothecae, were killed and dissected to identify possible endoparasitic stages. This occurred until 23 August; after this date, at least one parasite per host already extruded through the host cuticle, and we were able to reliably estimate prevalence without any dissection.

Behavior in aggregations was recorded by using tripod-mounted Sony TR425 Hi 8-mm camcorder positioned approximately 2 m from the aggregation (area 1, weeks 2 and 3). We observed the behavior of 32 female focal wasps already within three aggregations and 12 individuals after they joined an aggregation (2 min/wasps). There was no difference in activity between the three aggregations (ANOVA on arcsine transformed data, $F_{2,31} = 0.004$, $p = .99$) so these data were pooled.

To record the spatial behavior of stylized wasps in area 1, we marked 402 stylized wasps with enamel paint from aggregations on two mulberry trees and checked the same sites twice per week from 16 July–31 August. For wasps marked on the first five capture dates (up to 1 August, five cohorts), we constructed mark-recapture histories by using the program MARK (Cormack-Jolly-Seber open-population capture-recapture methods; reduced model of constant recapture probability; Lebreton et al., 1992). The use of individual covariates, such as parasite age, could not be included in the model without host dissection.

The presence on the nest of either parasitized adults or marked individuals from aggregations was estimated by the collection and dissection of adults from 21 nests over three dates (10 and 17 July and 3 August, $n = 7$ colonies each date). A further 12 nests were collected from 3–8 August and examined for the presence of marked wasps only. All nests were taken under roof tiles of the main building in area 1 early in the morning, before foragers had left the colony.

Laboratory behavioral recording of infected workers

In 2001 and 2002 *P. dominulus* nests, founded by queens from our study area, were maintained in the laboratory under 14-h light/10-h dark and 27°C–29°C standard conditions. The first instar strepsipteran larvae, which are the infective stage, were collected as they emerged live from adult female *X. vesparum* parasitic within stylized wasps that had overwintered (from the same area). Under a dissecting microscope, a single *X. vesparum* first instar larva was transferred by means of a single hair to the body of a larval wasp (the nest was cooled to 4°C so that the adult wasps could be removed first). The cell was marked with paint, and similar-sized larva were sham treated. Three nests in 2001 (nine infected and nine control workers in total; three each per nest) and eight nests in 2002 (12 infected and 14 control in total; between two and four per nest) were successfully infected. All nests had approximately 40 cells and less than eight workers at the time of infection,

that is, early “worker phase” (Reeve, 1991). Low replication per nest was owing to the difficulty of infecting brood, so we could not assess the colony effect.

To imitate a natural situation, infected nests were placed in small nest-box (simulating natural nesting inside cavities, volume = 500 cm³) and connected to large cages in which food, water, and paper for cell construction was placed (more than 12,000 cm³). In 2001 only the position of evidently parasitized and control workers was recorded three times/day (0730, 1130, 2330 h) for 3 days. In 2002 the behavior (time inactive; time off the nest; cell checking, a reliable indicator of work on the nest; and aggression received) of infected and control workers was recorded for the first 10 days from emergence (10 min/day per wasp; total observation time = 43 h 40 min). Observations were blind to the state of parasitism and conducted daily between 1400–1700 h.

We used the program MARK (www.cnr.colostate.edu/~gwhite/mark/mark.htm) for mark-recapture data analysis and SPSS for all other analyses. All tests are two tailed, and means are presented \pm SE. Where indicated, we performed transformation to approximately normalize the data. Logistic regressions were performed with Enter method.

RESULTS

Occurrence and composition of aggregations

A total of 91 aggregations ($n = 1322$ wasps, 99.99% *P. dominulus*), were checked from July–October: 97% of occupants were female, and henceforth only *P. dominulus* females are discussed. The first aggregation was observed on 16 July, the last on 10 October 2000. Before this, from 8 June–15 July, 17 stylozoid were captured singly and inactive on the underside of leaves (no uninfected wasps were found here). Two individuals were captured before 0700 h, that is, before foraging begins, indicating they had spent the night away from the nest. A further two individuals were captured flying around leaves, and again uninfected wasps were not found at this locality. All 19 wasps were presumably nominal workers; they had perfect wing condition and their tergites were not discolored as those of overwintered females are (Reeve, 1991). They contained five female adult *X. vesparum* that were not releasing first instar larvae (unlike overwintered ones which were observed) and 17 males at different stages of development. Adult free-living male *X. vesparum* were present on the 11 July, as evidenced by the capture of a wasp with two empty puparia.

In Tuscany, summer aggregations were usually found on the vegetation surrounding houses with high nest density. Wasps typically clustered under and between leaves of a few branches on prominent hedges and trees (*Molbus*, *Ficus*, *Hedera*, *Vitis*); *P. dominulus* males intensively patrolled the more exposed branches of the same trees in August and September. In area 1, many aggregations were observed on the lower branches of two large mulberry trees which were the sites of a previous long-term studies on *Polistes* male lek-territoriality at landmarks (Beani and Turillazzi, 1988, 1990). After 26 August, when the temperature abruptly decreased, aggregations were mainly found in sheltered sites, for example, inside creepers on buildings (*Hedera*, *Vitis*), eaves, interstices, and corners.

For July and August censuses, the mean size of 74 aggregations on vegetation was 12.93 ± 1.07 wasps, and this did not vary according to either area (seven areas, Kruskal-Wallis test, $H_6 = 3.48$, $p = .74$) or week (5 weeks, $H_4 = 5.03$, $p = .28$). The parasite prevalence, that is, the proportion of stylozoid females, was 0.98 ± 0.01 regardless of area of collection (logistic regression, Wald = 1.45, df = 6, $p = .96$) or week (Wald = 0.21, df = 1, $p = .88$). Twenty-seven wasps from

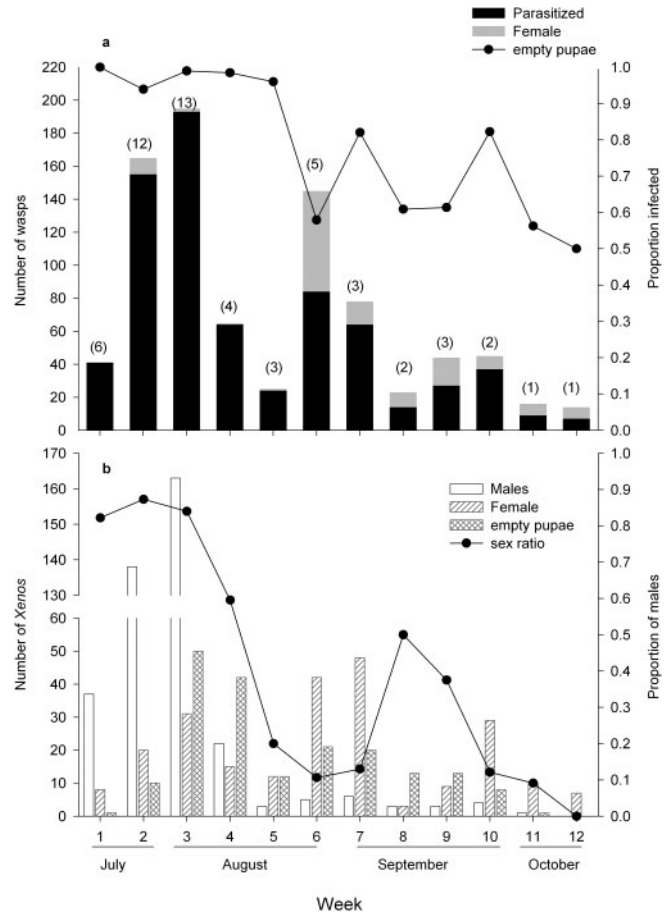


Figure 2

A survey of aggregations from area 1 over the course of the season. (a) The number of parasitized and unparasitized female wasps from aggregations according to the week of collection and the prevalence, that is, the proportion of infected individuals. Number of aggregations in parenthesis. The mean parasite prevalence was not affected by week when only wasps from weeks 1–5 or 6–12 were included in the model (Wald = 0.02, df = 1, $p = .88$ and Wald = 2.29, df = 1, $p = .14$ respectively). (b) The number of *X. vesparum* (including empty puparia) in aggregations according to week and sex ratio (proportion of males, not including empty puparia).

aggregations appeared unparasitized but after dissection were found to contain late endoparasitic stages of both sexes.

In area 1, where aggregations were continuously checked until October, the number of wasps and aggregations reached a peak at the beginning of August (Figure 2a). The mean parasite prevalence decreased from 0.98 ± 0.01 during the first 5 weeks to 0.64 ± 0.07 during 6–12 weeks. The likelihood of being parasitized decreased significantly between weeks 5 and 6 (odds ratio = 1.38, Wald = 84.15, df = 1, $p < .001$). This corresponded with the abandonment of mulberry trees. Thereafter, aggregations were found in more sheltered sites on buildings. The mean size of these late aggregations did not significantly differ from early ones (14.32 ± 0.03 versus 13.87 ± 0.02 wasps; $t_{54} = 0.01$, $p = .92$).

All aggregations in area 1 (Figure 2b), except week 12, included wasps infected by parasites of both sexes, mainly one per wasp (81%). The presence of adult male *X. vesparum* in the environment is indicated by a peak of 50 empty and 163 closed puparia at the beginning of August; a lower spike in September was owing to presence of empty puparia. The sex

Table 1

Mark-recapture analysis from area 1: probability of survival and recapture for wasps marked on a certain date (i.e., cohort)

Cohort date (<i>N</i>)	Proportion recaptured	Probability of survival	Probability of recapture
16 July (21)	0.38	0.97 ± 0.02	0.07 ± 0.04
24 July (55)	0.20	0.91 ± 0.03	0.07 ± 0.02
25 July (31)	0.10	0.88 ± 0.07	0.06 ± 0.05
28 July (31)	0.12	0.86 ± 0.09	0.06 ± 0.06
1 August (61)	0.28	0.93 ± 0.03	0.07 ± 0.02

ratio of *X. vesparum* was initially male biased, with a definitive shift toward females in late September–October; an increasing female bias was expected owing to the short male lifetime and the fact that only females overwinter. In the first week of August, 67 wasps in three aggregations were collected from the roof of an abandoned building in area 1, within interstices later used during diapause (not included in Figure 2a,b). These aggregations were characterized by a higher proportion of female parasites compared with ones on vegetation from the same week and area, (0.55 versus 0.16, odds ratio = 0.14, Wald = 37.15, df = 1, $p < .001$) and a lower parasite prevalence (0.68 versus 0.98, odds ratio = 0.07, Wald = 51.72, df = 1, $p < .001$).

Behavior of wasps within aggregations

Styloped wasps within aggregation did not, apparently, return to their nests, although at least until mid-August colonies were fully active. In area 1, none of 297 styloped wasps, which were marked and released from 16 July–7 August, were resighted on any of the 26 *P. dominulus* nests (801 females) collected during the same period within 200 m of the aggregation sites, that is, under the mean flight range of workers (Ugolini and Cannicci, 1996). Behavioral observations in the field have thus focused on extranidal aggregations on vegetation, in which 98% of wasps were parasitized. Wasps alighting on a leaf moved directly to the aggregation, joined it, and were subsequently inactive (mean time inactive/2 min = 0.88 ± 0.05). For individuals that had already aggregated, the proportion of time spent inactive was 0.97 ± 0.01 . Typical nest behaviors, such as aggression and trophalaxis, were not observed among aggregating wasps. Although within aggregations wasps were predominantly inactive, they did move between aggregations.

Of the 402 styloped wasps marked up to 31 August, only 50 were recaptured. For five cohorts examined in detail (Table 1), the probability of recapture of a marked wasp on subsequent censuses was very low, owing to spacing out of recaptures (not on successive days) and extensive movement among aggregations, rather than to removal of marked wasps from the population owing to mortality. In fact, the probability of survival until subsequent sampling was very high (Table 1), and some individuals were recaptured after a long time (29 days in three cases!). Although a high turnover of individuals existed among aggregations, the same leaves were occupied by aggregating wasps; in one case wasps were present on a single leaf on a mulberry tree for 36 days.

Behavior of artificially infected workers

To observe the spatial behavior of wasps, we divided laboratory cages into nesting and foraging areas. In the first experiment, nine wasps (infected by six male, three female *X. vesparum*)

left the colony, grouped together, and remained inactive inside the foraging area regardless of the sex of the parasite, whereas nine controls did not (proportion of time off nest, Mann-Whitney *U* test, $U = 45$; $n_1 = 9$, $n_2 = 9$; $p < .001$).

In the second experiment, both the position and behavior of 12 wasps (infected by nine male, three female *X. vesparum*) was compared with 14 controls. From the first day of adulthood until day 10, infected wasps were significantly more inactive than were controls (repeated-measures ANOVA, $p < .01$) (Figure 3a) and spent significantly more time off the nest than controls ($p < .001$) (Figure 3b). Infected wasps were never observed feeding brood, building cells, or foraging, that is, working, and checked cells less than did controls ($p < .001$) (Figure 3c). Infected wasps did not receive more aggression than did controls ($p = .09$, post hoc power test, $\beta = 0.63$, effect size = 0.8) (Figure 3d); in fact, the trend was for controls to receive more aggression.

All parasitized wasps deserted the nest and remained within the attached net. The mean date of departure, that is, the first day an individual spent 100% of its time off the nest, was 5.25 ± 0.93 days posteclosion, which was before the extrusion of the parasite through the cuticle of the host (8.7 ± 0.60 days). Controls only left the nest to forage. Nest departure before the extrusion of the parasite through the cuticle was also confirmed by the presence of 27 evidently nonparasitized wasps in aggregations that were dissected and found to contain endoparasitic final instar *X. vesparum* larvae with sclerotized mandibles (to aid in their extrusion through the cuticle): some were observed in the act of extruding during capture. Further, based upon nest collections we noted that very few *X. vesparum* extruded while their hosts were on the nest. Of the 894 adults dissected from 21 *P. dominulus* nests (area 1, 20/21 nests contained parasitized adults), 61 wasps were infected with 69 *X. vesparum*, and of those, only eight had extruded through the host cuticle (Fisher Exact test, $p < .0001$). Thus, less than 1% of adults on nests from a highly infected area were visibly parasitized (i.e., had extruded cephalothorax/cephalothecae of *X. vesparum*).

DISCUSSION

Colony desertion and subsequent extranidal aggregation formation by styloped *Polistes* females has here been extensively documented in the field and induced in the laboratory after the artificial infection of a social insect with a macroparasite. The occurrence of aggregations from seven sites over the first 5 weeks in which 98% of female *P. dominulus* were styloped is a priori evidence of parasite-mediated behavioral change (Figure 2a). Although the phenomenon is common (aggregations were noted also in 2001, 2002, and 2003 at our study sites, data not shown), this is the first example, to our knowledge, of a behavioral change induced by a parasite in *Polistes* wasps. In other social insects, colony desertion, but not aggregation outside the nest, may follow parasitism by nematodes, entomopathogenic fungi, trematodes, and parasitoids (Moore, 2002; Schmid-Hempel, 1998). The lack of attention hitherto given to strepsipteran parasitism of *Polistes*, despite its high prevalence in paper wasp colonies (Hughes et al., 2003), is owing to their cryptic nature (Figure 1b) and the absence of evidently parasitized adults on both field and laboratory nests: colony desertion occurred early in adult host life (mean = 5.25 days), before the parasite extruded through the cuticle. The subtle influence of this parasite has obvious implications for studies on the social behavior of *Polistes*; 31 other species have been recorded as hosts of Strepsiptera (Hughes, 2003).

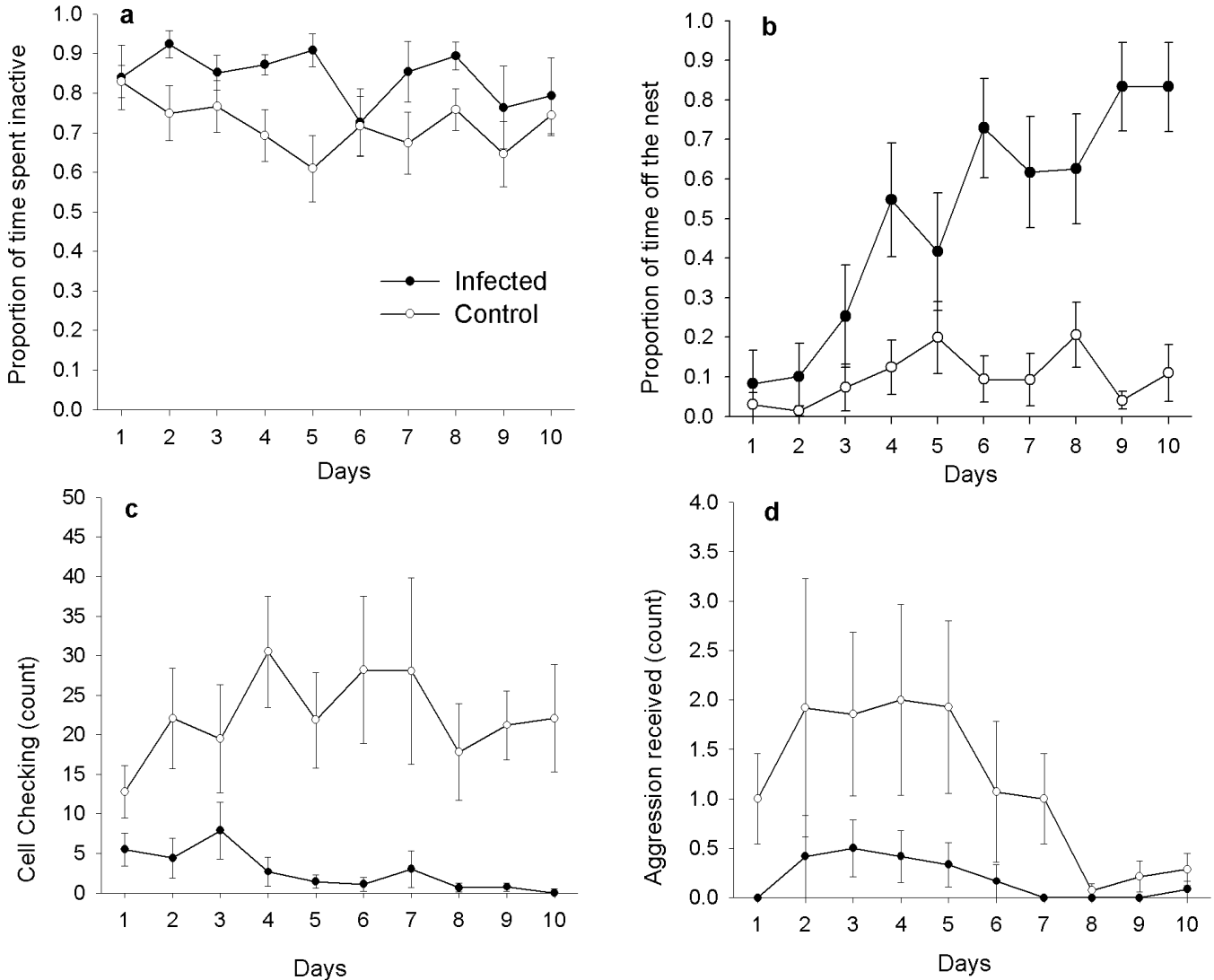


Figure 3

The behavior of artificially infected female wasps (filled circles) versus controls over the first 10 days of adult life. Parasitized wasps spent significantly more time inactive (repeated-measure ANOVA, $F_{1,24} = 7.57$, $p < .05$) (a); away from the nest ($F_{1,24} = 29.78$, $p < .001$) (b); checked cells significantly less ($F_{1,24} = 29.64$, $p < .001$) (c); and did not receive more aggression than controls ($F_{1,24} = 3.23$, $p = .09$) (d).

Aberrant aggregations

Styloped wasps deserting the field nests early in the season, as well as in our laboratory experiments, were nominal workers; that is, in the absence of parasitism they would have engaged in colony tasks. Nevertheless, determining the caste for styloped members of this primitively eusocial wasp is difficult because cues of caste—such as size, fat levels, ovarian development, and behavior with siblings—are all likely to be affected by parasitism. At the end of August (week 6), when the temperature notably decreased, the location of aggregations changed from exposed branches on trees to low sheltered vegetation and buildings, which were later adopted as hibernation sites. There was a significant decrease in parasite prevalence among aggregation occupants in area 1 (Figure 2a). This might be explained by the entry of healthy future queens, as these leave the nest to mate and aggregate outside, and may represent the transition from worker phase to prediapause aggregations. In addition, the shift from male- to female-biased sex ratio of the parasite (Figure 2b) suggests

that only wasps infected by female *X. vesparum* moved to hibernacula, whereas wasps containing empty puparia presumably died after male *X. vesparum* emergence, owing to their absence in extensive collections of overwintering healthy and infected wasps (data not shown).

Previous extranidal collections of female styloped *Polistes* (Hubbard, 1892; Pierce, 1909, 1918; Wheeler, 1910) do not mention aggregations. It is probable that high host density at our study sites promoted encounters among deserting wasps. Aggregation sites overlap spatially and temporally with either lek-sites (Beani, 1996) or prehibernation sites, which may signal some nonrandom spatial preference by infected and uninfected individuals. Aggregation formation follows from simple rules such as “stop when you encounter another individual” (Deneubourg et al., 2002) and could explain their patch distribution (only some branches of a tree or a portion of a roof eave had been selected as an aggregation point). Aggregations were characterized by extreme inactivity of wasps (97% time inactive), high turnover (Table 1), and strong site attachment (in area 1 a particular leaf was occupied for

36 days). (A high turnover and inactivity are not mutually exclusive as behavioral observations focused on within-aggregation behavior rather than a daily time budget, which would have included interaggregation movement.) The defensive capabilities of *Polistes*, combined with their aposematic coloration, should promote aggregations (Guilford, 1990), which are common in paper wasps at the end of the reproductive phase (Reeve, 1991; Turillazzi, 1980; West-Eberhard, 1969). To date, aggregations of stylopized social insects are known only for the genus *Polistes*. These “aestivation/hibernation gatherings,” first noted by W.D. Hamilton (e-mail to Laura Beani, 21-3-1999, 11:20 hrs; both e-mails stored at W.D. Hamilton Archive, in preparation at the British Library, material available by appointment. See Summers A, Leighton John J, 2001. The W.D. Hamilton Archive at the British Library. *Ethology, Ecology and Evolution* 13:373–384) may be an example of exploitation of the preexisting gregarious behavior and a way to reduce extrinsic mortality outside the nest during final parasite development.

Why do stylopized wasps leave the nest?

Although aggregations of stylopized females are surprising, the salient point is that parasitized females deserted the nest both early in their adult life and in the season. For *Polistes*, nest departure normally occurs for sexuals (males and future queens) to secure matings while workers remain on the nest to gain indirect fitness benefits through work (but for desertion of healthy workers for direct fitness, see Reeve et al., 1998). Stylopized females cannot achieve direct fitness as they are physiologically castrated (Strambi and Strambi, 1973), so may be expected to remain on the nest and gain indirect fitness benefits through work. In the present study, infected individuals were not observed to work (Figure 3c), and this corresponds to the general lethargy reported for infected *Vespa* (unpublished data cited in Matsuura and Yamane, 1990). Therefore, why do infected wasps leave the nest? Desertion is undoubtedly associated with the presence of Strepsiptera and may benefit the parasite or the host or simply be a “boring by-product” of infection (Dawkins, 1990; Moore, 2002; Poulin et al., 1994). Within this framework, we explore some explanations for the nest desertion by *P. dominulus* females after infection by Strepsiptera.

Desertion owing to eviction by siblings of lazy and costly workers was not observed, and the trend was for stylopized individuals to receive less aggression than did controls (Figure 3d). The time spent on the nest, by stylopized individuals, was characterized by extreme inactivity (Figure 3a). Desertion to satisfy the nutritional needs of the developing parasite, or stressed host, appears unlikely as the nest is a rich source of food, whereas lek-sites only occasionally have resources (Beani, 1996). Stylopized wasps were observed maxillating prey items and imbibing larval secretions; the latter are 50 times more rich in amino acids than is flower nectar (Hunt et al., 1982). In fact, desertion occurred soon before the extrusion of the final instar and after a period of maximal growth. Desertion as an altruistic act to reduce infection to kin would appear a good general strategy for infected social insects but is untenable in this case because a female *X. vesparum* is infective only if inseminated and copulation appears not to occur on the nest (see below); moreover, wasps parasitized by both sexes of the parasite desert the nest. In bumblebees infected by conopid flies, nest desertion at night retards parasite development (the nest is thermoregulated; Muller and Schmid-Hempel, 1993). *Polistes* nests, which are not thermoregulated, did not show a similar pattern in desertion times. In the later stages of conopid infection, the

host deserts the nest completely and, through aberrant “digging” behavior, promotes overwintering survival of the conopid pupa (Muller, 1994). In both host groups, obvious parallels exist with the final desertion of the colony by the infected individual occurring to promote parasite life-cycle completion.

In line with Hamilton’s attention to parasites, we here hypothesize that nest desertion and aggregation by stylopized wasps is an example of adaptive parasite manipulation of host behavior in order to facilitate parasite mating. Copulation is unlikely to occur on the nest as free-living males are vigorously attacked by occupants (when stylopized wasps are constrained to remain close to the nest until male emergence; Hubbard, 1892; Hughes D, personal observation). Moreover, the short adult stage (less than 5 h) of male *X. vesparum* means that males would have to emerge at the exact time a neotenic female, parasitic within a host on a nest, is both present and ready to be inseminated. Mate encounter is enhanced away from the nest, at aggregations. The peak of the mating period, as indicated by the maximum number of empty puparia, was in week 3 (Figure 2b), which corresponded with the peak in both aggregation and stylopized wasp number (Figure 2a). Although male Strepsiptera are generally considered extremely rare, they can be found close to aggregations. We observed five volant *X. vesparum* males and one mating on a leaf 20 cm from an aggregation. We also attracted seven males to a caged receptive female (data not shown). In addition, assuming copulation could take place on the nest, the fecundity of female *X. vesparum* is more than 3000 first instars per female (data not shown), so although remaining on the nest affords vertical transmission opportunities, the high probability of injurious superparasitism (at any one time there are less than 50 larval wasps/nest) has probably selected for nest dispersal and increased horizontal transmission.

Anecdotal evidence suggests that stylopized workers of *Vespa* are inactive in the colony (Matsuura and Yamane, 1990) and that stylopized ants display positive phototropism and elevation seeking on vegetation outside the nest (Cook, 1996; Ogloblin, 1939). The date of departure from the nest by infected *Polistes* (5.25 days) probably reflects a balance between staying on the nest to gain food for parasite development and leaving to reduce the risk of attacks towards lazy and costly nest mates, or some hitherto unknown cost related to the extrusion of the parasite through the host cuticle. Thus, we suggest that nest desertion occurs to facilitate parasite reproduction.

In conclusion, infection of *Polistes* females by Strepsiptera results in a parasite-mediated behavioral change that we suggest is adaptive to the parasite for life-cycle completion, although specific tests to evaluate the costs and benefits of nest desertion need to be carried out. Parasite-induced changes in host behavior range from the relatively subtle (Poulin and Latham, 2002) to the incredibly complex (Eberhard, 2000). In the case of stylopized wasps, their behavior is aberrant because it expressed out of context, both spatially and temporally: colony desertion by workers and summer aggregations of castrated wasps at leks. Finally, our data has pertinence for the debate concerning the role that parasites play in the evolution and maintenance of social behavior. It has been strongly claimed (O’Donnell, 1997) that parasitic castrators, particularly Strepsiptera in *Polistes*, reduce intracolony conflict over reproduction and may promote social behavior, *sensu lato* the “subfertility hypothesis” (West-Eberhard, 1975). Here we demonstrate that stylopization, rather than promote sociality, actually results in early nest desertion by infected individuals.

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