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Research article

Prevalence of the parasite Strepsiptera in *Polistes* as detected by dissection of immatures

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Summary. Though the paper wasp genus, *Polistes,* is well studied, we know little of the incidence of parasitism in this group. Here we present details of 45 nest dissections for 4 species: *P. dominulus* (Christ), *P. gallicus* (L.), *P. stabilinus* Richards and *P. carnifex* (F.) to detail levels of parasitism of colony members by the obligate parasitic group of insects, the Strepsiptera. All 4 species showed evidence of parasitism among immature members. For 3 species, more than 50% of inspected nests were parasitized and the levels of parasitism among brood (larvae and pupae) was very high and did not differ significantly between parasitized nests. One species, *P. stabilinus,* suffered very low levels of parasitism, which may be related to its habitat choice. The number of parasites per host was positively related to the proportion of infected brood (parasite prevalence) and in some cases reached phenomenally high levels, which casts doubt on previously assumed mechanisms of infection for nest-making Hymenoptera, i.e. phoresy. We also document cases of egg parasitism and encapsulation in *Polistes* nests. Our data show that parasitism levels greatly varied among areas. Finally, the recent debate on the competitive advantage of *P. dominulus* in its introduced range, USA, has credited an absence of strepsipteran parasites of this species in facilitating its spread. For the first time, we document levels of parasitism for this species in its nature P range and this would appear to corroborate previous claims. We place our work in the context of other studies of parasitism of social insects and posit that the genus *Polistes* may have much to offer to this field.

Key words: *Polistes, Xenos,* Strepsiptera, parasitism, encapsulation.

Introduction

Our understanding of social behaviour has benefited greatly from the use of *Polistes* wasps for a number of reasons: their position on the social spectra, open accessible nests facilitating observation, small colony size and tractability to laboratory manipulation (Reeve, 1991). Yet despite claims of some members of this genus, i.e. *Polistes dominulus*, being the "most well studied social wasp" (Queller et al., 2000), we know disappointingly little of ecological factors affecting the colony. Particularly, we know little of the abundance of parasites and pressures imposed through parasitism.

Studies of parasitism in *Polistes* have concentrated mainly upon incidence and probable effects of parasitoids belonging to Hymenoptera, Lepidoptera and less commonly Diptera (reviewed in Nelson, 1968; Makino, 1985; Yamane, 1996). These ectoparasitoids which oviposit on the cell walls, typically attach to pupal hosts and consume it, after which they pupate and leave. Some lepidopterans are purely predaceous, tunnelling from cell to cell consuming meconia and host tissue (i.e. families Cosmopterigidae, Tineidae and Pyralidae, see Rau, 1941; Jeanne, 1979; Miyano, 1980; Strassmann, 1981). The impact of parasitoids is the loss of workers or sexuals through destruction of brood (Miyano, 1980; Strassmann, 1981; Makino, 1989; Makino and Sayama, 1994) and resistance is through increased adult awareness to ovipositing parasitoids ("parasite dance", West-Eberhard, 1969), and "multiple comb" construction (Jeanne, 1979). Knowledge of the parasites of paper wasps has received distinctly less attention than for other social insects (see Nelson, 1968 and Appendix 2 of Schmid-Hempel, 1998).

In an effort to increase our knowledge, we document the abundance of the obligate parasitic insect group, Strepsiptera. Despite being long known to occur in *Polistes* (Rossi, 1793 in *P. dominulus*), they have never been the subject of ecological studies. Previous work is either anecdotal (e.g. Bohart, 1942), incidental observations arising out of host centred studies (e.g. Pardi, 1946; Khalifa, 1953; Turillazzi, 1980), taxonomic notes (e.g. Brues, 1903; Kifune, 1979; Cook and Mathison, 1997), or physiological alterations due to parasitism (Strambi et al., 1982). Our most thorough knowledge has come from a study of late season nests (Octo-

ber/November) where of 11,542 male *P. annularis* L. collected, less than 5% were infected (Dunkle, 1979). Appreciation of Strepsiptera among students of *Polistes* is minimal; a review of *Polistes* (Reeve, 1991) and two specifically of nest symbionts and parasites (Nelson, 1968; Yamane, 1996), make no mention of them. However, two recent reports do mention Strepsiptera in *Polistes* and posit that the spread of *P. dominulus* in the USA, where it was introduced, may be partly explained by an inability of native strepsipterans to use it as a host (Pickett and Wenzel, 2000; Gamboa et al., 2002).

Our aim in this study is, through a series of nest collections and dissections, to document parasitism by Strepsiptera for 4 species of *Polistes* from both Old World (Italy, *P. dominulus* and *gallicus*), and New World habitats (Mexico, *P. stabilinus* and *carnifex*). We concentrate upon immature members, as these are the susceptible members of the population. Additionally, information of parasitien for adults on the nest is difficult to obtain due to nest departure by parasitized individuals (see below). The laborious nature of our approach dictates reduced sample sizes.

Natural history of Strepsiptera belonging to the genus *Xenos*

The Strepsiptera are an order of obligate parasitic insects which have been found from such disparate hosts as: Thysanura, Blattodea, Orthoptera, Mantodea, Hemiptera, Diptera and the Hymenoptera. Those infecting *Polistes* belong to the genus *Xenos* (Family Stylopidae). Permanently endoparasitic adult females undergo, diapause in adult female wasps and once this is completed, release the infective stage, 1st instar larvae. These motile, free-living instars emerge live from the anterior region of the female (brood canal opening in the cephalothorax) which protrudes through the intersegmental membranes of the hosts' abdomen. They are released onto flowers and are transferred to a nest by a foraging wasp (phoretic transport, Hughes unpublished data), though direct transport when a wasp parasidized by a nature female, lands on the nest cannot be excluded (this study). They enter the larval hosts within the nest and undergo hypermetamorphosis to larviform 2nd instar endoparasitic larvae. They remain as successive endoparasitic stages until the host attains adulthood, at which time they exert their anterior region through the host cuticle to form pupae if male, or adult stage if female (the latter do not pupate, Kathirithamby, 2000). Males emerge from pupae and fly off to find a mate (longevity < 5 hours) and hence mirror parasitoids, though the host is not "killed as a developmental necessity" (Godfray, 1994). Females are true parasites and remain associated with the host through adulthood. Sperm transfer takes places through the females' anterior region and sperm is released into the haemocoel where fertilisation takes place. (See general reviews by Kinzelbach, 1978; Kathirithamby, 1989).

Parasitism by Strepsiptera is cryptic: a significant proportion of the lifecycle is spent as an endoparasite and adult females are often difficult to see (the protruded area is dorso-ventrally compressed and lies between the host tergites or sternites, requiring close inspection to locate). The most commonly encountered stage is the male pupa, which is visible as a large rounded pupal mass protruding through the hosts' abdomen. In addition, parasitized wasps depart the nest soon after emergence from the cell and before the neotenic adult or pupal stage parasites are visible (Hughes et al., in prep.), thus reducing encounters with *Polistes* researchers. Strassmann (1981) noted that, of the tens of thousands of *P. exclamans* Viereck observed by her, only 8 were parasitized.

Materials and methods

Nest collection

In Italy, 12 nests of *P. dominulus* and 10 nests of *P. gallicus* were collected from 9 areas (A-I, Table 1) surrounding Florence (43°45′N, 11°18′E). Of the 22 nests: 7 came from external walls of houses, 6 were from candle holder of tombs in a cemetery, 4 from the inside of plant pots, 3 from cavities of free standing external walls and the remaining two from the stems of plants. Nests were collected between the 8th and 26th June 2000 after 0900 hrs. The possibility that some adults were away from the nest cannot be discounted, thus the number of adults was not included in statistical analysis. Nests are believed to be pre- or early post-emergence of workers, based upon collection date, brood and cell number. In Area C, for *P. dominulus*, the 2 very large nests (170, 119 cells, Table 1) were reused nests from the previous year.

In Mexico, 15 nests of *P. stabilinus* and 8 nests of *P. carnifex* were collected from 3 areas surrounding the 'Los Tuxtlas Biological Station', Veracruz State (18°35′N95°5′W) which lies within a 700 ha area of primary and secondary rain forest. The collection sites were: buildings or plants of the field station (Area X, Table 1), a 3 km^2 patch of forest surrounding the station (Y) and buildings and plants of a village 2 km far from the station (Z). Nests were collected between the 27th April and 13th May 2001 after 0900 hrs. These two species differed greatly in habitat choice: *P. stabilinus* nests were only found on heavily shaded plants within rain forest (notably the palm *Astrocaryum mexicanum* Liebm.). By contrast, *P. carnifex* was found on sunlit buildings or plants along the edge of paths. In Veracruz, seasonality in nesting exists (R. Jeanne, pers. com.), and based upon cell and brood number counts, nests were assumed to be around worker emergence phase. For *P. carnifex* a nest of 7 cells was found which might represent a re-foundation event.

Dissection and parasite detection

Collected nests were sacrificed on the day of collection and all adults and brood (larvae/pupae) subsequently dissected in 75% alcohol using a stereomicroscope (X40). Eggs were visually checked for parasitism (the parasite is easily seen so dissection is not needed). All dissections were carried out by one person to avoid observer bias. Strepsipteran 1st instars enter larval wasps at any point on the body including the head capsule and/or mandibles of larvae, so that these parts must be checked. Detection of 1st instars/shed exuviae (which are dark brown) is relatively easy, both for eggs and brood. Recently moulted 2nd instar detection is more difficult owing to their small size and translucent appearance that is not dissimilar from host tissue, except their black eyespots. Older and larger 2nd instars are more yellow and easy to see, as well as 3rd instars.

A host (egg, brood member or adult) was said to be parasitized if a 1st instar, exuvia or any later stages were observed inside. An exuviae was not counted when a corresponding later stage was present, thus the same parasite was not counted twice. Besides the percentage of infected nests for each species, we considered the 'parasite prevalence' (Poulin, 1998, p. 30), i.e. the proportion of infected individuals (larvae and pupae) per nest. The exact number of eggs was unfortunately not

recorded for all nests, so parasite prevalence for eggs is not calculated, but egg parasitism only occurs in exceptional circumstances (see Results, 1c). Only one adult of 145 in total was parasitized (a female *P. stabilinus*), so parasite prevalence was not calculated for adults. 'Parasite load' is a loosely defined term (Schmid-Hempel, 1998, p. 289); here it is the number of parasites that entered the host divided by the total number of brood in the nest.

Encapsulation

Encapsulation is a common insect cellular defence against foreign bodies, such as parasites, which involves surface attachment by host haemocytes until a capsule is formed and the parasite is killed (Tanada and Kaya, 1993). The capsule melanizes after a few hours. We counted capsules only if the 1st instar (or its shed exuviae) was discernable within the capsule. Encapsulation did not affect the evaluation of parasite prevalence, as later instars were always observed in hosts at the same time (due to multiple parasite entry). Encapsulated 1st instars were counted to determine parasite load. In the case of *P. carnifex*, where two hosts had extremely high parasite loads, the true levels of encapsulation (and parasite load) could not be accurately determined (the host tissue coalesced because of contamination of the dissecting dish with water and prevented accurate dissection and parasite counting).

Statistical analysis

Count data were log transformed and proportions arcsine transformed to approximately normalize them. If a normal distribution was not produced an alternative non-parametric test was employed (Zar, 1999). The effect that area has upon parasitism cannot be examined quantitatively due to the insufficient number of replicates per area. A heuristic examination of the data was undertaken in this instance. Means are given ± SE. All statistics tests are two-tailed.

Results

1a. Ubiquity of parasitism

Of the 4 species examined (Table 1) the overall percentage of infected nests was: 58% for *P. dominulus*, 60% for *P. gallicus*, 50% for *P. carnifex* and 13% for *P. stabilinus* (Fig. 1a). For the Italian hosts (*P. gallicus* and *dominulus)* the strepsipteran was *Xenos vesparum* Rossi*,* whilst for *P. carnifex* the strepsipteran was identified as a new species (Kathirithamby and Hughes, in prep.), and for *P. stabilinus* only endoparasitic stages were found so the species could not be determined.

Table 1. Parasitism by *Xenos* spp. in immatures from nests of 4 *Polistes* spp. Column 1, N = samples size of nests/area. The values in subsequent columns are for single nests. Total and means ± SE in parenthesis are for the values in columns 2–5 (all nests, parasitized nests only and unparasitized nests only). The squared brackets show the number of infected brood per nest. The symbols after the square brackets are: * = nest in which encapsulation in some brood was found (see Results), † = nest where egg parasitism occurred (see Results), [a] = below true value (see Methods)

Species	Area (N)	All adults/nest	Cell/nest	Brood/nest [Parasitized brood/nest]	No. of *Xenos* nest
P. dominulus	A (2)	1, 1	32, 28	13[0], 15[0]	0, 0
(Italy)	B (2)	2, 9	21, 88	7[1], 38[1]	1, 1
	C (3)	10, 11, 1	170, 119, 23	58[2], 31[3], 5[0]	2, 3, 0
	D (1)	1	45	14[0]	0
	E (3)	4, 2, 2	44, 26, 27	30[23]*, 16[7]*, 16[7]	49, 10, 9
	F (1)	1	29	18[0]	0
Total (mean ± SE)		*45 (3.75 ± 1.12)*	*652 (52.67 ± 13.98)*	*261 (21.75 ± 4.34)*	*75 (6.12 ± 4.03)*
Parasitized nests	(7)	*40 (5.71 ± 1.55)*	*495 (10.71 ± 21.60)*	*196 (28.0 ± 6.44)*	*75 (12.34 ± 7.50)*
Unparasitized nests	(5)	*5 (1.0 ± 0.0)*	*157 (31.4 ± 3.70)*	*65 (13.0 ± 2.17)*	–
P. gallicus	B (2)	1, 1	40, 27	16[0], 12[0]	0, 0
(Italy)	C (1)	1	29	5[4]	7
	G (2)	7, 3	48, 44	22[8], 13[0]	15, 0
	E (3)	1, 3, 7	14, 37, 58	7[5], 24[9], 15[13] †	9, 13, 84
	H (1)	3	40	6[0]	0
	I (1)	4	56	34[6]	6
Total (mean ± SE)		*31 (3.1 ± 0,74)*	*393 (39.30 ± 4.26)*	*154 (15.4 ± 2.88)*	*134 (13.40 ± 8.04)*
Parasitized nests	(6)	*23 (3.83 ± 1.10)*	*242 (40.33 ± 7.00)*	*107 (17.83 ± 4.50)*	*134 (22.34 ± 12.41)*
Unparasitized nests	(4)	*8 (2.0 ± 0.58)*	*151 (37.75 ± 3.70)*	*47 (11.75 ± 2.10)*	–
P. stabilinus	X (2)	3, 3	43, 67	27[0], 48[0]	0, 0
(Mexico)	Y (13)	2, 4, 7, 2, 5	26, 26, 58, 18, 59	9[3], 23[0], 33[0], 11[0], 36[0]	3, 0, 0, 0, 0
		7, 1, 1, 1, 4	75, 72, 17, 17, 37	41[0], 35[0], 6[0], 13[0], 35[0]	0, 0, 0, 0, 0
		3, 2, 16	22, 18, 115	1[0], 6[0], 67[2]	0, 0, 2
Total (mean ± SE)		*61 (4.07 ± 0.99)*	*671 (44,73 ± 7,48)*	*400 (26.67 ± 4.6)*	*5 (0.33 ± 0.22)*
Parasitized nests	(2)	*18 (9.0 ± 7.0)*	*141 (70.5 ± 44.5)*	*76 (38.0 ± 29.0)*	*5 (2.5 ± 0.5)*
Unparasitized nests	(13)	*43 (24.92 ± 3,97)*	*530 (40.7 ± 6.33)*	*324 (24.92 ± 3.97)*	–
P. carnifex	X (5)	2, 3, 1, 1, 1	7, 29, 55, 33, 15	2[2] †*, 15[3]*, 19[0], 23[3]*, 3[1]	40[a], 2, 0, 6, 23
(Mexico)	Z (3)	1, 1, 1	11, 12, 8	6[0], 8[0], 1[0]	0, 0, 0
Total (mean ± SE)		*11 (1.37 ± 0.26)*	*170 (21.25 ± 5.89)*	*77 (9.62 ± 2.95)*	*72 (10.29 ± 5.84)*
Parasitized nests	(4)	*7 (1.75 ± 0.48)*	*84 (21.0 ± 6.05)*	*43 (10.75 ± 5.63)*	*72 (18.0 ± 8.55)*
Unparasitized nests	(4)	*4 (1.0 ± 0.0)*	*86 (21.50 ± 11.20)*	*34 (8.50 ± 3.80)*	–

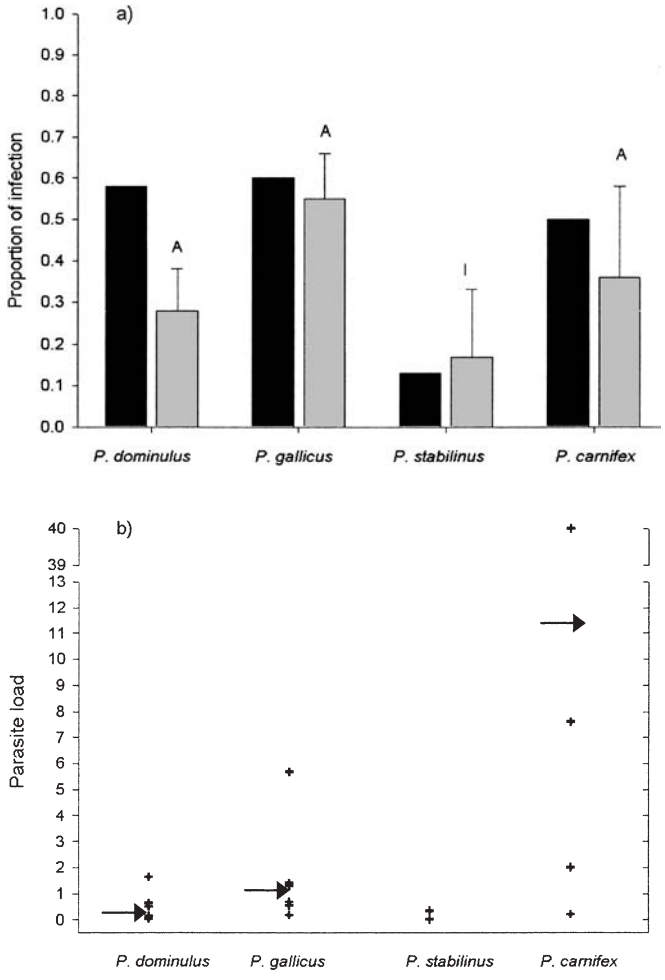

Figure 1. a) The proportion of nests (N = 45) infected for 4 species of *Polistes* (black bars) and the mean (± SE) parasite prevalence (number of parasitized brood/nest) for infected nests only (grey bars). The three species indicated by the letter A did not significantly differ (Kruskal-Wallis test), *P. stabilinus* was not included (see text). b) The parasite load (number of parasites/brood) of the infected nests for the 4 species of *Polistes*. The arrows indicate the mean value (except for *P. stabilinus* where only two samples exist)

Strepsipteran parasites were found in all stages of the host: eggs, larvae, pupae and adults. The brood contained mostly 2nd instars and recently entered 1st instars, very rarely 3rd instars. Eggs contained only 1st instars whilst the one parasitized adult contained a 3rd instar. We usually found more than one developmental stage in the same nest and in the same larvae/pupae, because multiple entry was frequent.

1b. Among brood (larvae and pupae)

The mean parasite prevalence per nest (proportion of infected larvae and pupae) was greater for *P. gallicus* than for *P. dominulus* (0.33 ± 0.11 vs. 0.16 ± 0.07 respectively), though this difference was not significant (Paired t test: $t_{20} = -1.138$, $p = 0.26$). In Mexico, the mean parasite prevalence among brood members was significantly higher for *P. carnifex* (0.18 ± 0.13) than for *P. stabilinus* (0.02 ± 0.02) due to the occurrence of only two parasitized nests for the latter in our sample (Mann-Whitney U-test: $U = 36$, $N_1 = 8$, $N_2 = 15$, $p < 0.05$). For each species there was no apparent relationship between brood number and parasite prevalence (Spearman rank correlation: *P. dominulus*, $r_s = 0.35$, n = 12, ns; *P. gallicus*, $r_s = -0.56$, n = 10, ns; *P. stabilinus*, $r_s = 0.05$, N = 12, ns and *P. carnifex* $r_s = 0.17$, n = 12, ns). Neither was there a relationship between number of cells and parasite prevalence (P. *dominulus,* $r_s = -0.06$, ns; *P. gallicus*, $r_s = 0.09$, ns; *P. stabilinus*, $r_s = 0.22$, ns and *P. carnifex* $r_s = -0.14$, ns). However, any relationship might be masked as we could not control the area, due to our limited sample. When only parasitized nests of the 4 species were included (n = 19), then the mean parasite prevalence among brood approximately doubled for each species. Except for the less parasitized *P. stabilinus* (which was not included due to low sample size) there was no difference in parasite prevalence in infected nests among the 3 species (Kruskal-Wallis test: $H_2 = 2.73$, $N_1 = 7$, $N_2 = 6$, $N_3 = 4$, $P = 0.25$, Fig. 1a).

The parasite load (the average number of parasites that entered per brood, Fig. 1b) occasionally reached phenomenally high levels: 5.67 in one nest of *P. gallicus*, 7.6 in one nest of *P. carnifex*, and in a further nest a value of at least 40.0 was found (this is below the true value, as not all parasites could be counted, see Methods). For the first example the nest was within a candle holder of Area E and 5cm close to a dead *P. dominulus* female parasitized by a female *X. vesparum,* from which 1st instar larvae were still emerging. A similar event may have occurred for *P. carnifex*: both nests were exposed and a female *P. carnifex* with an adult female *Xenos* sp., which was releasing 1st instars, was collected flying within 1m of one of these nests. The exposed location of the nests and high parasite load suggested that a parasitized female alighted on the nest.

In infected nests the range of parasites per brood member was very variable from: 1–9 (*P. dominulus*), 1–15 *(P. gallicus*), 1–40 (estimate, *P. carnifex*) whilst in *P. stabilinus* we only ever found 1 parasite per brood member (therefore, here parasite load equalled parasite prevalence). In nests with a high prevalence, i.e. where many larvae and pupae were infected, the parasite load was also high (Fig. 1a, b). For the Italian species, a significant positive relationship between parasite load and prevalence was evident (Linear regression: $r^2 = 0.909$, $F_{1,11} = 100.43$, $P < 0.001$ for *P. dominulus* and $r^2 = 0.792$, $F_{1,9} = 30.52$, $P < 0.001$ for *P. gallicus*). A similar trend was observed for *P. carnifex,* but because, in one case, the value recorded was below the true value, an analysis was not performed.

1c. Among adults and eggs

None of the adults on the nest at the time of collection were parasitized: *P. dominulus* (n = 45), *P. gallicus* (n = 31), *P. carnifex* (n = 61). For *P. stabilinus*, one female among 115 adults from one nest was parasitized by a 3rd instar *Xenos.* In a nest of *P. carnifex*, two adults contained encapsulated 1st instars (indicating successful defence, see below).

Eggs were found to be parasitized for two species of wasps (*P. gallicus* and *carnifex*). For the former species, in the nest with a high (5.67) parasite load (Area E), 6 of the 22 eggs contained a total of ten 1st instars that had not moulted. In a similar fashion the heavily infected *P. carnifex* nest, with a parasite load of at least 40.0, one of the four eggs contained three unmoulted 1st instars within it.

2. Encapsulation

Two of the four species of wasps encapsulated strepsipteran parasites. For *P. carnifex* encapsulation was seen in all four parasitized nests and all infected brood contained capsules. Notably, the two hosts with high parasite load were filled by capsules, but their exact number was not accurately determined. For another nest we observed, within a single pupa, 23 encapsulated exuviae and the same number of 2nd and 3rd instars, i.e. moulting and subsequent growth had occurred despite encapsulation of the exuviae. In *P. dominulus* all three nests from Area E (Table 1) contained encapsulated *X. vesparum.* Within these nests 71%, 40% and 30% of infected brood had some form of encapsulation (Table 1). In this species we recorded the following 18 encapsulation events: 1st instar larvae (33%, n = 6), moulting 1st instar (11%, n = 2) or exuviae (67%, n = 10). All hosts which had encapsulated 1st instars also had apparently healthy 2nd instars (due to multiple parasite entry). The category 'moulting 1st instar' was assigned when, at the time of sacrifice, a 2nd instar was emerging from its exuvia which was partially encapsulated. This was observed in two larvae of two nests of *P. dominulus,* and whether this represents successful defence or not is unclear. Where the exuvia was encapsulated, the subsequent endoparasitic stage, either 2nd or 3rd, was unmolested and apparently healthy.

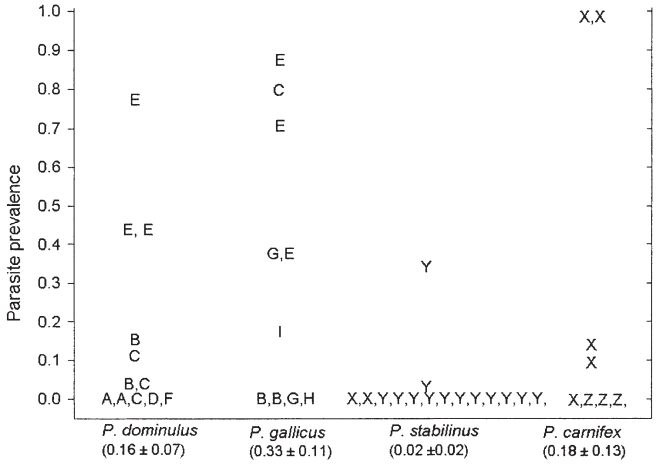

Figure 2. A descriptive plot of parasite prevalence in 45 nests of 4 *Polistes* species, according to the areas of collection, labelled with different letters (see Table 1 and Methods). The overall parasite prevalence ± SE for the species is within the parenthesis

3. Factors affecting parasitism

Area

In Fig. 2 the parasite prevalence for each of the 45 nests examined was plotted according to its area of collection. This heuristic analysis indicated a patchy distribution of parasites. For Italy, Area E had a high prevalence for both *P. dominulus* and *gallicus* (three nests each), and here we recorded encapsulation in the former, egg parasitism and high parasite load in the latter (Table 1). These phenomena were not recorded in Area B (two nests of each species), which had a low overall parasitism (Table 1). In a similar vein, those nests of *P. carnifex* collected around the station (Area X) were mostly parasitized (4/5) and all these had evidence of encapsulation, whilst egg parasitism was observed for one nest.

Discussion

The present study is the first to systematically collect and dissect whole *Polistes* nests on order to evaluate parasitism by Strepsiptera. Our chief findings are the apparent ubiquity and the high levels of parasitism by the genus *Xenos*. All four species of *Polistes* were parasitized to some extent. In *P. dominulus, gallicus* and *carnifex,* at least 50% of nests were parasitized. Within these species the prevalence of parasitism, i.e. the proportion of infected brood, was high (0.16–0.33), and particularly so when only parasitized nests were considered (0.28–0.55). No significant difference in prevalence was observed among parasitized nests of the three species (nor did these nests differ in size, i.e. number of cells or number of brood). The parasite load, number of parasites which entered/per host, had a highly significant relationship with parasite prevalence (for the Italian species). That is, nests with a high proportion of parasitized brood had more parasites per brood member. By contrast, only 2 of 15 *P. stabilinus* nests were infected and its prevalence/load of parasitism was low. *P. stabilinus* might not be a target species of *Xenos* (here the species of Strepsiptera was unidentified). Alternatively, the low level of infection might reflect its nesting habits. Nests widely dispersed within rain forest could obviate both the aggregation of foraging wasps at central points (where infective agents can be picked up, see Schmid-Hempel, 1998, p. 231), and the incidental discovery of nests by parasitized wasps.

Having established that parasitism exists in previously unappreciated levels, our data also draws attention to the mode of infection for colonies. The apparently bimodal distribution of *Xenos* per nest (1–3 in 7 cases, 10 or more in a further 7 cases, see Table 1) supports the hypothesis of two main infection methods: phoretic transport, i.e. a few *Xenos* 1st instars attaching to the abdomen of *Polistes* wasps at flowering patches (Hughes, unpubl. data), and/or their direct and massive release close to combs from a wasp infected by a mature *Xenos* female. The exceptionally high parasite load observed for two nests of *P. gallicus* and *carnifex*, and a few records of parasitized wasps close to nests (see Results, 1b), suggest that the second mechanism might occur. All work

concerning hymenopterans' nests parasitized by Strepsiptera have assumed phoresy as the only mechanism of infection and two studies documented it (for non-social species, Linsley and McSwain 1957; Maeta et al., 2001). Pardi (1946) first observed that gynes parasitized by Strepsiptera moved from one nest to another during the pre-emergence period (though not establishing if they were infective). This weak association of parasitized gynes with early colonies has been recently observed both in laboratory and in the field, where 3 *P. dominulus* bigynic nests were collected with one parasitized female on the back of the nest (L.Beani, unpubl. data). J. Strassmann (pers. comm.) observed parasitized *P. dominulus* wasps, with adult female *X. vesparum*, close to pre-worker emergence nests (whether they were infective is unknown).

Whilst we did not investigate the effect colony size had upon parasitism levels, we speculate that larger nests may be less liable to parasitism as the arrival of a larvipositing female would be prevented by attendant wasps. But conversely, an increased number of attendant wasps would promote parasitism through phoretic infection pathways. Previous work (Strassmann, 1981; Müller and Schmid-Hempel, 1992) has shown that larger colonies suffer higher levels of parasitoidism, but nest size had no effect on social parasitism (Gamboa, 1978). The relationship between colony size and parasite level is controversial (see Schmid-Hempel, 1998).

Parasitism of nests appears to be influenced by area (Fig. 2), suggested also by Dunkle for *P. annularis* (1979). Variation of parasitism levels with area is a common phenomenon (Combes, 2001), for example in bumblebees (*Bombus terrestris* L.*)* parasitized by conopid flies (Schmid-Hempel and Schmid-Hempel, 1996) and by intraspecific parasitism in *P. metricus* Say (Gamboa, 1978). An area effect, indicated by our heuristic analysis, is substantiated by the occurrence of high prevalence /load and cases of egg parasitism and encapsulation in some areas and not others.

The other significant findings of this work are the occurrence of egg parasitism and encapsulation. Both, to our knowledge, are first time recordings for *Polistes*. For strepsipterans infecting solitary of hymenopterans that provision and close the cell before their egg has hatched (e.g. andrenid bees, eumenid wasps), it is the normal method of entry (Linsley and McSwain, 1957; Maeta et al., 2001 respectively). As *Polistes* nests usually contain larvae, unless very early or very late in the cycle, entry into eggs is puzzling. It was found in nests of *P. gallicus* and *P. carnifex* which had a very high parasite load among the brood (5.67 and 40.0 respectively, Results, 1b) and may reflect an adaptation of the parasite to avoid larval hosts which are very heavily parasitized. An additional instance (not in Table 1) was an apparently refounded nest, 12th June 2000, of *P. gallicus* with eight eggs, one of which was parasitized. It is not known if parasites entering eggs can complete their lifecycle but because of the apparently common practice of oophagy in *Polistes* (see Karsai et al., 1996), it would appear not to be the optimal strategy, but rather a best of a bad job scenario.

Host defence by encapsulation occurred in three nests of *P. dominulus* and four nests of *P. carnifex* (all nests from the same area, respectively). Studies of *Drosophila melanogaster* Meigen have shown that encapsulation is costly (Kraaijeveld and Godfray, 1997). In bumblebees, activating the immune system with artificial 'parasites' is costly and cannot be undertaken effectively in stressful conditions (Köning and Schmid-Hempel, 1995), leading to increased mortality (Moret and Schmid-Hempel, 2000) and immune investment for subsequent brood (Moret and Schmid-Hempel, 2001). Whether local adaptation by hosts or mistaken entry by the parasite sub-species into a non-target host is the cause of encapsulation (e.g. Paxton and Pohl, 1999), the fact is that some larvae (and hence colonies) are expending resources on defence. More importantly, it is not always successful as some parasites moulted to the 2nd instar and grew, despite their exuviae being encapsulated.

Finally, our data has pertinence for the current debate on *P. dominulus* spread in the USA (Cervo et al., 2000; Pickett and Wenzel, 2000; Gamboa et al., 2002). A collection of 1,123 *P. dominulus* by Pickett and Wenzel (2000) showed none were parasitized by Strepsiptera whilst 16–19% of adult *P. fuscatus* (n = 112) were infected (based on field collections of colonies and subsequent rearing, K. Pickett pers. com.). The high parasitism levels inside its home range, Italy, compared to an absence of parasitism outside (New York state, USA), suggests that an absence of adapted parasites in the latter area aids in its spread and apparent competitive advantage (Pickett and Wenzel, 2000).

An increasing body of work has focused upon parasitism (reviewed in Poulin, 1998; Combes, 2001) and its impact upon social insects (Currie, 2001; reviewed in Schmid-Hempel 2001, 1998). The genus *Polistes* may serve as a further model for this approach. The factors which make *Polistes* attractive to researchers may facilitate studies of parasitism (Reeve, 1996) and Strepsiptera would appear a prime candidate because, unlike the parasitoids of *Polistes,* it forms an association with the host from the larval to adult stage. Our data show that parasitism is widespread, occasionally at very high levels and in some case elicits host immune responses. *Polistes* larvae (and colonies) may offer a further example to study ecological immunology (Sheldon and Verhulst, 1996). In conclusion, it is hoped that this previously unappreciated and enigmatic group, in combination with the amenable host genus, *Polistes,* may add a further dimension to studies of parasitism in social insects.

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