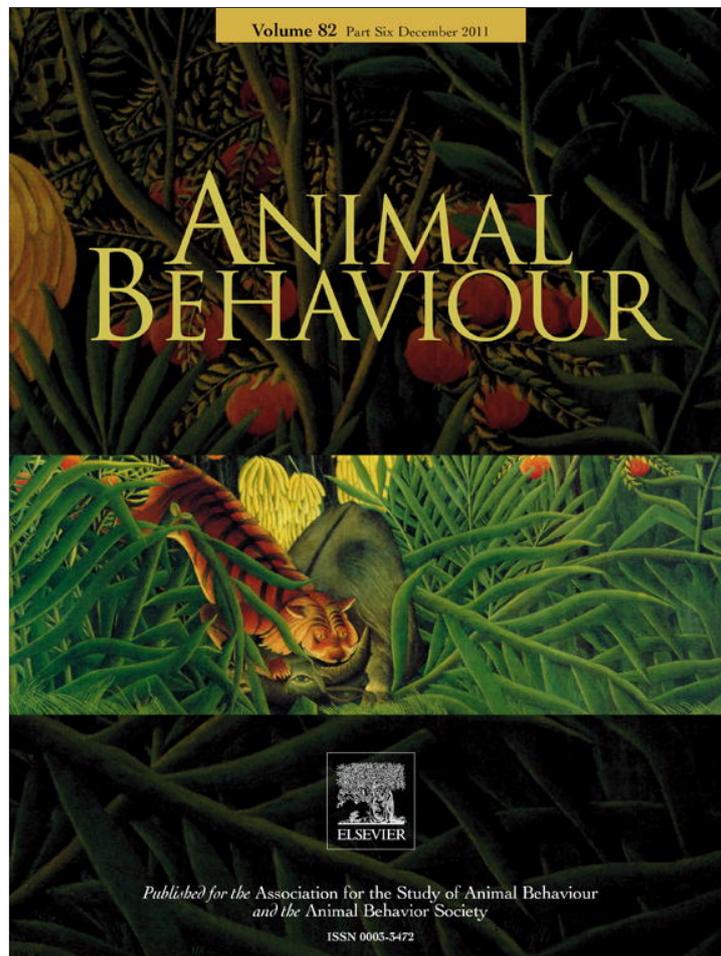


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When a parasite breaks all the rules of a colony: morphology and fate of wasps infected by a strepsipteran endoparasite

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The macroparasite *Xenos vesparum* affects both the behaviour and the physical traits of its host, the social wasp *Polistes dominulus*. Female wasps, if parasitized, do not perform any social tasks and desert the colony to gather at specific sites, where the parasite mates; at the end of summer they form prehibernating clusters joined by healthy future queens to overwinter. Parasitized wasps become highly gregarious. In April, healthy wasps leave the aggregations to found new colonies, while parasitized wasps remain in overwintering groups and release parasites to infect wasp larvae only later in the season. We studied the prolonged gregarious behaviour of parasitized wasps and analysed the morphology of parasitized and healthy wasps in aggregations collected over a 7-year period to determine whether the parasite affects host size, wing symmetry, ovarian development and lipid stores. All parasitized wasps were smaller and had undeveloped ovaries and more wing fluctuating asymmetry than unparasitized wasps, irrespective of time of year, parasite load and parasite sex. If infected only by one or two *X. vesparum* females, the wasps had large fat bodies, which could facilitate their overwintering. In contrast, wasps infected by at least one male parasite had little lipid and died at the end of the summer. Thus, *X. vesparum*, may play a role in the fate of its host, by exploiting wasps' tendency to form aggregations outside the colony and by altering its caste system, nutrient allocation, diapause timing and life span to achieve its own reproduction and dispersal.

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Insect colonies represent rewarding targets for parasites, owing to the considerable amount of stored resources and the density of potential hosts. However, 'breaking into the fortress' is not an easy matter (Schmid-Hempel 1998) and many species have evolved a complex set of colony defence mechanisms to counterbalance the increased risk of parasite spread (Cremer et al. 2007). Nests of the primitively eusocial paper wasp *Polistes dominulus* (Hymenoptera, Vespidae) are only relatively protected as they are small, not sheltered by any envelope and clustered on buildings, although larvae are well defended by adult nestmates. *Xenos vesparum* (Strepsiptera, Xenidae) is an obligate endoparasite of temperate *Polistes* species (Hughes et al. 2004a) and *P. dominulus* is its primary host.

Overwintering wasps bearing fertilized *Xenos* females may spread the infective first-instar larvae, the triungulins, while foraging on flowers (phoresy) or wandering among nests (Hughes et al. 2003) prior to the emergence of the first workers and in the middle of the day, when the queen is foraging and the nest is unprotected (Beani & Massolo 2007). This is a seasonal and daily 'undefended time window' also exploited by social parasites, that is, species that profit by the parental care of a congeneric species to rear their brood (Nannoni et al. 2001; Ortolani & Cervo 2009). To escape defensive reactions of the adult wasps, triungulins rapidly penetrate into all larval stages of *Polistes*, one or more per wasp larva, without any selective host-seeking behaviour (Vannini et al. 2008; Manfredini et al. 2010a). The immune response of the host is delayed and ineffectively directed against the exuvia of the triungulin (Manfredini et al. 2007).

Among the morphophysiological changes induced by the parasite, the most dramatic is the castration of the female wasp (Strambi & Strambi 1973; Strambi et al. 1982). Stylopization of the host, that is, the protrusion of the parasite's male puparium or female cephalothorax from the host's abdomen (Fig. 1a), is detectable 5–10 days after host emergence (Hughes et al. 2004b).

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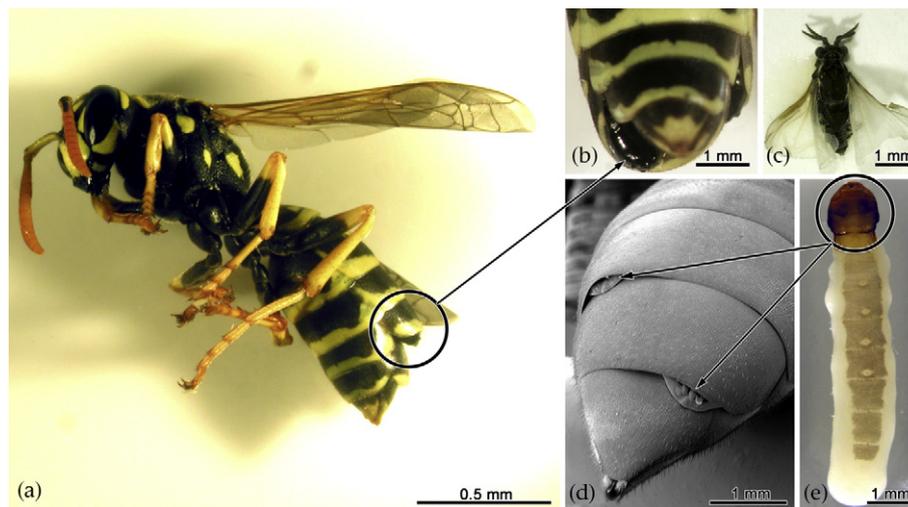


Figure 1. (a) Superparasitized *P. dominulus* female wasp. (b) Two puparia protruding from the wasp's abdomen. (c) *Xenos* male. (d) Two flattened cephalothoraces, detectable between the wasp's tergites. (e) *Xenos* neotenic female.

At this time, parasitized female wasps have already deserted their colony, where they did not perform any social task, to cluster in aberrant summer aggregations (first observed by W. D. Hamilton in several species of *Polistes*; Hughes 2002). Here, the free-living winged *X. vesparum* males break their pupal cap and abandon their hosts to fertilize the permanently endoparasitic female (Beani et al. 2005). A *Xenos* male dies within a few hours of emergence, whereas a female may survive until the next spring, well protected inside the abdomen of a female wasp.

The aim of this long-term study was to assess the influence of *X. vesparum* on the phenotype of *P. dominulus* females. We focused on wasp overwintering aggregations (Dapporto et al. 2006a; Cini & Dapporto 2009), comprising both infected and uninfected *P. dominulus* females, during 7 years. First, we hypothesized that parasites subtly exploit the gregarious behaviour of the host, here, the clustering tendency of wasps outside the colony, to survive until spring: we expected that styloized wasps should initiate aggregations when colonies fall down, in August, and that they lengthen this gregarious phase of host inactivity until spring. Second, we expected the parasite load to affect more than one physical parameter of its host, owing to the nutritional depletion that the host faces before reaching adulthood (Giusti et al. 2007) and the inhibition of juvenile hormone-secreting organs (Strambi & Girardie 1973). Therefore we compared body size, fat bodies and ovarian development, which are critical parameters for caste determination and survival in paper wasps, between styloized and healthy wasps. Moreover, we carried out a preliminary evaluation of *Polistes* wing fluctuating asymmetry, that is, deviations from a perfect bilateral symmetry. Finally, we assessed the effect of *Xenos* males and females on physical condition and life span of the host: owing to the extreme sexual dimorphism of strepsipterans (Grimaldi et al. 2005), we expected a different exploitation of host resources.

METHODS

Wasp Collection

Aggregations (hereafter five individuals or more in very close contact) of parasitized and unparasitized *P. dominulus* female wasps were collected from two areas of Tuscany (Impruneta, Florence; San Gimignano, Siena) for a period of 7 consecutive years

(2004–2010) and across three aggregation phases: (1) mid-August/mid-September (late summer, i.e. colony decline), $N = 1233$ wasps, 42 prehibernating aggregations, sheltered on vegetation or buildings; (2) January (winter diapause), $N = 1282$, 37 hibernating aggregations, under roof tiles, often on old nests; (3) last week of March/mid-April (early spring, i.e. foundation phase), $N = 1006$, 43 posthibernating aggregations from the same roofs. We selected mixed aggregations to compare parasitized and unparasitized wasps that were used as controls from the same group and site. Owing to the difficulty of collecting a large sample of parasitized specimens, we combined aggregations from different years; there was no evidence of any significant difference in the size of aggregations or in the physical traits of the wasps between years (see Results). Small sample sizes precluded between-year testing of wing asymmetry and parasite load. We also sampled one to four wasps settling close to (5–50 cm) aggregations (hereafter called scattered). Moreover, we sampled dead wasps from hibernating and posthibernating collections.

Clustering Pattern and Wasp Morphology

We evaluated the proportion of parasitized and dead wasps across the three aggregation phases and the number of aggregated and scattered wasps. We labelled the protruded parasite as male (Fig. 1b) in the presence of a puparium (although flying males usually emerged from their puparia before the dates of collections, Fig. 1c) and as female (Fig. 1d) in the presence of a cephalothorax (for details on the life cycle see Kathirithamby 1989, 2009; Beani 2006). In mid-August wasps are likely to be visibly styloized (Hughes et al. 2004b). A subset of *Xenos* females (48 from prehibernating, 28 from hibernating and 23 from posthibernating collections) was dissected to check for the presence of triungulins.

A total of 425 wasps from mixed aggregations ($N = 244$ parasitized and $N = 181$ unparasitized) were carefully dissected. We measured the size in a subset of 352 wasps (193 parasitized and 159 unparasitized) without knowing the wasp's infection status. Wings were flattened against a glass slide to facilitate measurement. We chose the length of the medial cell of the right forewing (see Fig. 5 in the Results) as a good indicator of wasp size because it correlates positively with the whole length of the wing (Pearson correlation: $r_{350} = 0.726$, $P < 0.001$) and the head width ($r_{350} = 0.735$, $P < 0.001$). We also compared the length of the right and the left

forewing medial cell in 137 parasitized and 81 unparasitized wasps collected from the same clusters, to investigate the wing fluctuating asymmetry (FA = Right (R)–Left (L)) regardless of any possible abrasion of the wing tip. In a trait exhibiting 'true' FA (Polak 1993a), variation of R–L values should be normally distributed (Kolmogorov–Smirnov test: $Z = 0.082$, $N = 218$, $P = 0.88$). We evaluated wasp size and the absolute value of FA in relation to occurrence, number and sex of the parasites. We divided our parasitized individuals into six categories: infected by one male, two males, one female, two females, one male and one female, three parasites or more.

For the 425 dissected wasps we scored ovaries and lipid storage. We considered ovaries to be developed (rank 3) if bearing at least one mature or nearly mature oocyte at the base of the ovary, with yolk and about the size of an egg already laid (see Strassmann et al. 2004) as well as reabsorbed oocytes (Gobbi et al. 2006). Rank 2 ovaries contained easily detectable oocytes under development, whereas rank 1 ovaries were undeveloped, thread-like, with filamentous ovarioles (Fig. 2a, b, c). We scored fat bodies under the third tergite, which was rarely occupied and deformed by a protruding parasite: rank 1 if monolayered, rank 2 if multistratified and rank 3 if they contained masses of adipocytes (Fig. 2d, e, f).

All the statistical analyses were conducted using SPSS 13.05 (SPSS Inc., Chicago, IL, U.S.A.). Values are given as means \pm SE. We tested for normality and equality of variance in wasp size data. When we compared measurements across 7 years, three aggregation phases and parasitism, we used ANOVA and the Tukey honestly significant difference (HSD) test as a post hoc test for parametric data and the chi-square test, Mann–Whitney U test and Kruskal–Wallis test (with post hoc multiple comparison test) for nonparametric data.

RESULTS

Aggregations

Prehibernating aggregations were located in August on specific flowering plants and bushes, patrolled as nuptial arenas by *P. dominulus* males (Beani 1996), and then on buildings, where they were hidden under eaves, corners, creepers (*Hedera*, *Vitis*, etc); the

same buildings were later selected by wasps hibernating under roof tiles, often on abandoned nests and inside beam cracks. As a general rule, aggregations were not randomly located but were close to each other (less than 1 m apart) in the same sites, year after year. The mean number of wasps for each aggregation was 25 ± 2.76 in late summer, 32.73 ± 4.22 in winter and 11.38 ± 1.33 in early spring ($N = 121$). Prehibernating and hibernating aggregations were significantly larger than posthibernating ones (Kruskal–Wallis test: $H_{2,119} = 27.64$, $P < 0.001$; post hoc multiple comparison test: $P < 0.001$), regardless of the year of collection ($H_{6,115} = 5.33$, $P = 0.502$).

In prehibernating collections we found that half of the wasps were parasitized (Fig. 3). Noticeably, the mean ratio of healthy wasps increased from 30.43% to 50.17% in 13 mixed aggregations sampled twice at the same site between mid-August and mid-September in 2009: there were significantly fewer healthy wasps than parasitized ones in August aggregations, whereas in September their proportions were similar (two-tailed unpaired t tests: $P = 0.006$ and 0.09 , respectively). In prehibernating collections, wasps were infected by parasites of both sexes (Fig. 4), although in mid-August most *Xenos* males (77.98%) had already emerged (386 empty and 109 closed puparia). We found triungulins inside the brood canal in only three of 48 dissected *Xenos* females. Hibernating and posthibernating samples were mainly composed of healthy specimens (90% and 79.2%; Figs 3 and 4); parasitized wasps were usually infected by one *Xenos* female (80.47% and 87.56%, respectively) containing adipocytes, oocytes and embryos but no mature triungulins (51 dissected samples), that is, their full development was delayed until spring.

Gregarious Behaviour

We compared the ratio of aggregated versus scattered wasps for parasitized and unparasitized specimens through the three aggregation phases (Fig. 3). Stylopized wasps were usually aggregated (five or more individuals in close contact, see Methods), whereas healthy wasps were aggregated like stylopized ones only in large hibernation groups (chi-square test: $\chi^2_1 = 1.11$, $P = 0.181$). In late summer stylopized wasps were already aggregated in large prehibernating clusters whereas a quarter of healthy wasps were still

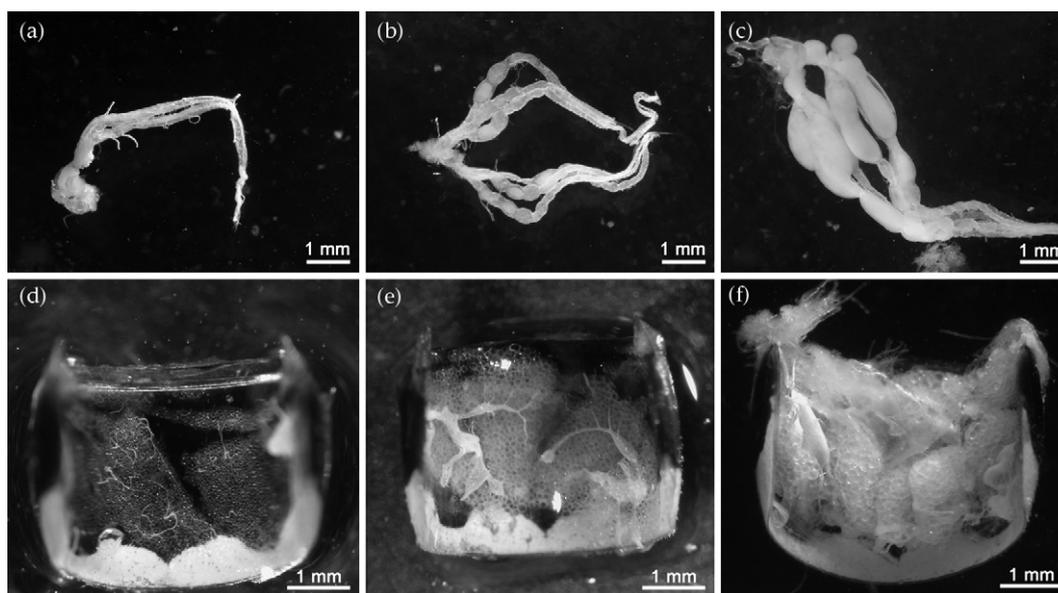


Figure 2. Levels of (a, b, c) ovarian development and (d, e, f) fat body storage in *P. dominulus*: (a) thread-like ovaries, with filamentous ovarioles bearing no visible oocytes (rank 1); (b) small ovaries bearing detectable oocytes under development (rank 2); (c) large ovaries, bearing nearly mature/mature oocytes located in the base of the ovary (rank 3); (d) inside of the third tergite with monolayered adipocytes (rank 1), (e) multistratified adipocytes (rank 2) and (f) abundant fat bodies (rank 3).

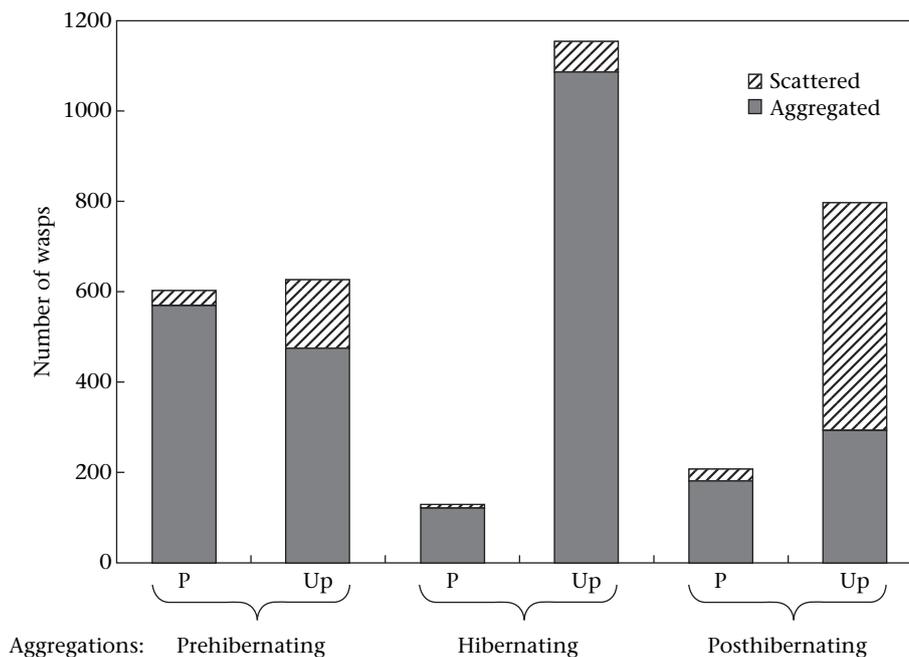


Figure 3. Wasp spatial behaviour across the three aggregation phases. Aggregated = five or more individuals in close contact; scattered = one to four individuals, close to (5–50 cm) aggregations. P = parasitized; UP = unparasitized.

scattered on vegetation and walls ($\chi^2_1 = 81.79$, $P < 0.001$). This asymmetry in spatial behaviour was more noticeable in posthibernating groups ($\chi^2_1 = 107.6$, $P < 0.001$): 87.6% of stylotized wasps were still aggregated under roof tiles, while healthy wasps were mostly scattered (63%), singly or in groups of two to four under roof tiles. Often, the tile selected as a shelter was later the nesting site for one or more foundresses (personal observation). The ratio of dead to live wasps under tiles was not significantly different between parasitized and unparasitized ones, both in January (8.15% versus 7.23%; $\chi^2_1 = 0.045$, $P = 0.705$) and in March/April (11.06% versus 7.11%; $\chi^2_1 = 3.416$, $P = 0.056$).

Wasp Size

Parasitized wasps were significantly smaller than unparasitized ones (ANOVA: $F_{1,351} = 34.59$, $P < 0.001$). There was a noticeable size overlap between the two groups: lower and upper ranges for wing

medial cell measurements were 5.9–9.1 mm in parasitized wasps versus 6.8–9.7 mm in unparasitized ones. Wasps sampled in prehibernating aggregations were smaller than those collected in hibernating and posthibernating ones, the difference between August/September and March/April aggregations being the highest (ANOVA: $F_{2,350} = 4.67$, $P = 0.01$; Tukey HSD: $P = 0.018$), regardless of the year of collection ($F_{6,346} = 0.61$, $P = 0.726$). Nevertheless, the effect of the parasite (Fig. 5) remained highly significant across months (two-factor ANOVA: $F_{5,347} = 8.31$, $P < 0.001$; aggregation phase: $F = 3.28$, $P = 0.039$; parasitism: $F = 25.13$, $P = 0.001$). Wasp size was not affected by either the number or the sex of the parasite (ANOVA: $F_{5,187} = 0.48$, $P = 0.789$).

Wing Asymmetry

Fluctuating asymmetry, here evaluated by comparing the lengths of right and left forewing medial cells (Fig. 5, inset), was

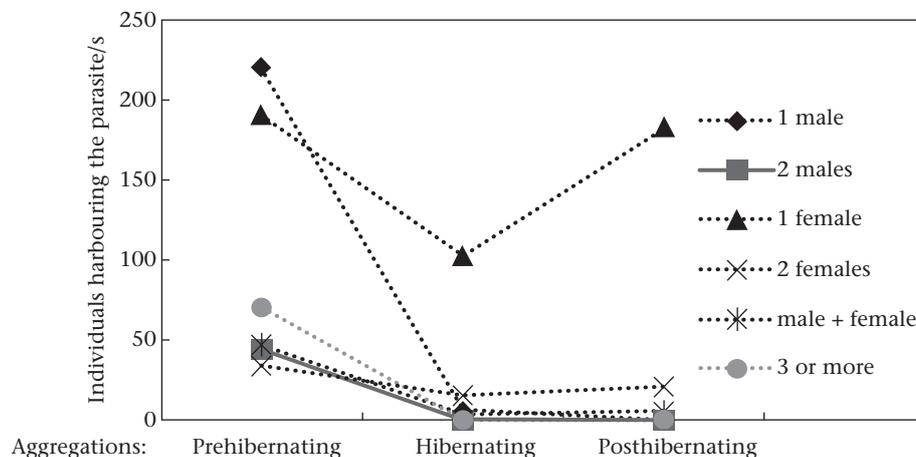


Figure 4. Parasite prevalence (sex and number of *X. vesparum*) in parasitized wasps from prehibernating, hibernating and posthibernating aggregations.

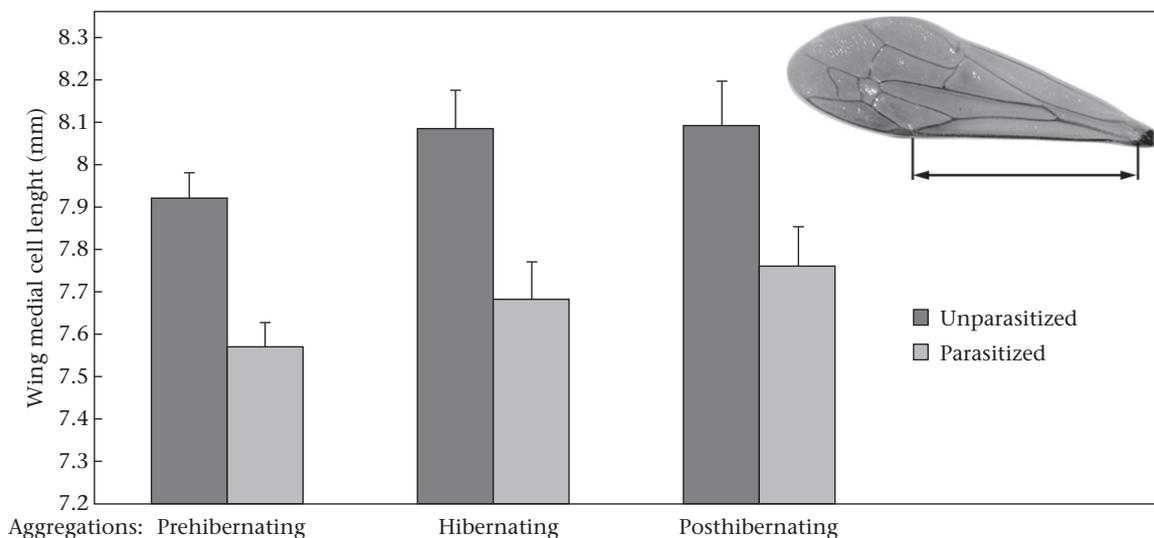


Figure 5. Body size comparison, measured by the length (means \pm SE) of the forewing right medial cell (see inset), between parasitized and unparasitized wasps across the three aggregation phases.

significantly higher (ANOVA: $F_{1,217} = 9.23$, $P = 0.003$) in parasitized wasps (mean difference = 0.17 ± 0.01 mm, $N = 137$) than unparasitized wasps (mean difference = 0.07 ± 0.01 mm, $N = 81$). We verified that there was no significant correlation between the whole length of the wing and FA values (Pearson correlation: $r_{216} = 0.054$, $P = 0.425$) to exclude any confounding effect of a possible correlation between the magnitude of the trait and the degree of symmetry. Although the difference between right and left forewings was particularly relevant in wasps infected by three parasites or more (mean difference = 0.21 ± 0.05 mm, $N = 20$), wing asymmetry was not influenced by the number or the sex of *Xenos* (ANOVA: $F_{5,132} = 0.81$, $P = 0.547$).

Ovary Development

All parasitized wasps had undeveloped ovaries (rank 1), regardless of aggregation phase (Kruskal–Wallis test: $H_{2,242} = 0.78$, $P = 0.678$) and parasite load (Kruskal–Wallis test: $H_{5,242} = 1.03$, $P = 0.960$). Ovary development across the three aggregation phases was more variable in unparasitized wasps, ranging from rank 1 to rank 3 (Kruskal–Wallis test: $H_{2,179} = 20.61$, $P < 0.001$; Fig. 2). It was significantly lower in the prehibernation phase (post hoc multiple comparison test: $P < 0.01$), whereas there was no evidence of any significant difference in healthy wasps from hibernation and posthibernation collections. The difference between parasitized and unparasitized wasps was highly significant in all the aggregation phases (Mann–Whitney test: prehibernating: $U = 5192$, $N_1 = 176$, $N_2 = 108$, $P < 0.001$; hibernating: $U = 45$, $N_1 = 30$, $N_2 = 32$, $P < 0.001$; posthibernating: $U = 133$, $N_1 = 38$, $N_2 = 41$, $P < 0.001$). There was no evidence of any significant difference between the different years of collection (Kruskal–Wallis test: $H_{6,419} = 3.89$, $P = 0.691$).

Fat Bodies

Regardless of the year of collection (total sample: $H_{6,419} = 2.94$, $P = 0.817$), unparasitized wasps had lower lipid stores across the months (Kruskal–Wallis test: $H_{2,179} = 13.57$, $P < 0.001$; Fig. 6). Lipid stores in stylotized specimens were parasite dependent. In prehibernation collections, where the parasite load was highly variable, fat body allocation (Figs 2 and 6) was significantly affected by

the occurrence, the sex and the number of *Xenos* parasites (Kruskal–Wallis test: $H_{6,282} = 102.1$, $P < 0.001$). Wasps infected by one or two females had higher lipid stores than those infected by one or two males (post hoc multiple comparison test: $P < 0.001$ and $P = 0.017$, respectively) or by three or more parasites ($P < 0.001$) and were not significantly different from unparasitized wasps in prehibernating aggregations. In hibernating and posthibernating collections, where parasitized wasps mainly included specimens infected by one or two *Xenos* females (Fig. 4), the degree of fat storage was lower in parasitized than unparasitized wasps, but this difference was weak in winter aggregations (Mann–Whitney test: $U = 349$, $N_1 = 30$, $N_2 = 32$, $P = 0.04$) and not significant in spring (Mann–Whitney test: $U = 692$, $N_1 = 38$, $N_2 = 41$, $P = 0.363$).

DISCUSSION

Gregarious Behaviour

The presence of *X. vesparum* influenced the gregarious behaviour of *P. dominulus* outside the nest in three ways. First, at the peak of the colony cycle, they formed aberrant extracolony aggregations in early summer (described in the same population by Hughes et al. 2004b). Second, they were the focal point of prehibernating aggregations, owing to the settlement of healthy gynes in sites already selected by infected wasps (presumably marked long term with venom and cuticular peptides, Turillazzi et al. 2006a, b). Third, they not only initiated, but also lengthened the overwintering gregarious phase (Fig. 3): in early spring stylotized wasps were still aggregated while healthy individuals began to scatter to found new colonies. If they act as infection vectors to the next generation of the host, waiting for the full development of triungulins and mature *Polistes* nests in sheltered inactive groups could be advantageous for the parasite. An analogous 'sit and wait strategy' has been described for colony usurpation by conspecifics (Starks 1998), as well as by social parasites (Cervo & Dani 1996). An increased diapause period is in agreement with the time arms race between the parasite and its host during infection.

Desertion of the colony to form lengthened extracolony aggregations differs from the completely novel behaviour outlined by Baer & Schmid-Hempel (1999) in several host–parasite systems, because unparasitized wasps also continue to be gregarious when

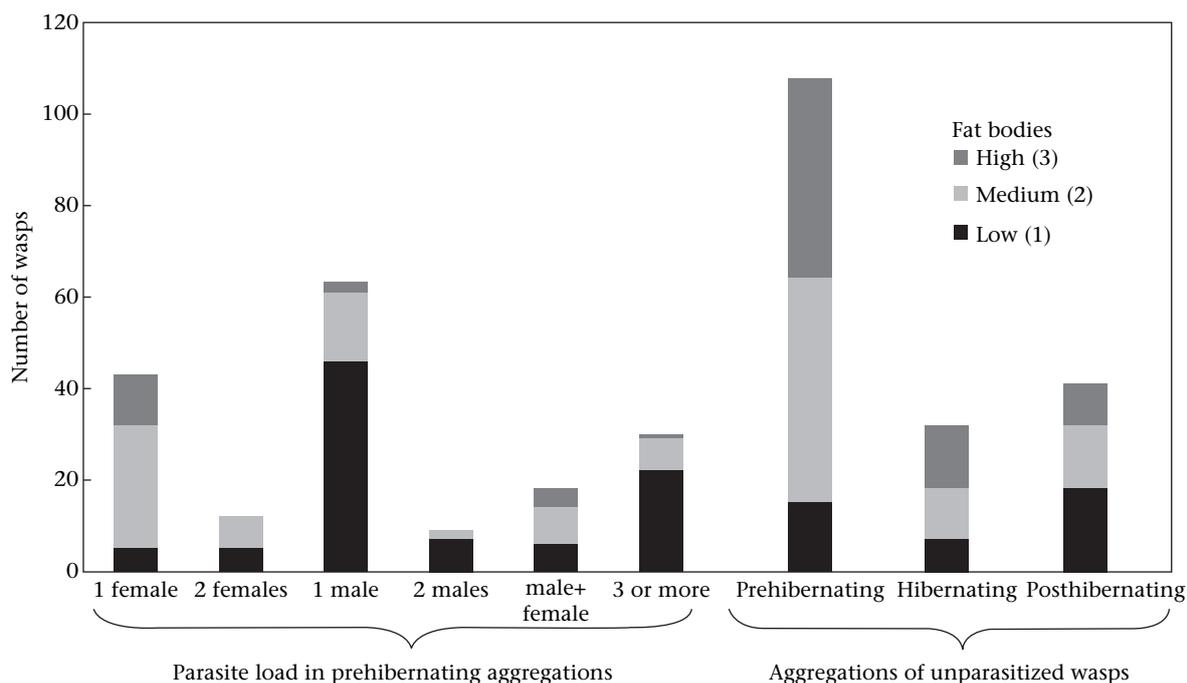


Figure 6. Parasite load (sex and number of *Xenos*) in relation to lipid storage (high = rank 3; medium = rank 2; low = rank 1; see Fig. 2) in 176 parasitized wasps collected from prehibernating aggregations and 181 unparasitized wasps collected from prehibernating ($N = 108$), hibernating ($N = 32$) and posthibernating ($N = 41$) aggregations.

the colony declines and styloped females remain motionless (Hughes et al. 2004b) and do not get involved in either aggressive behaviours or trophallaxis (Dapporto et al. 2006b). Noticeably, the percentage of dead wasps in hibernating and posthibernating aggregations was low among both parasitized and unparasitized specimens. Gregarious behaviour is likely to enhance the survival of the wasps at low temperatures by increasing the temperature of the aggregation as well as through increase in the antimicrobial effects of dominulin A and B, two recently described peptides on the cuticle and in the venom of *P. dominulus* wasps (Turillazzi et al. 2006b); indeed, a high microbial activity may be linked to group size (Hoggard et al. 2011). Moreover, clustering may enhance the aposematic signals typical of many aculeate hymenopterans: styloped wasps are well equipped with an efficient sting apparatus and active venom, although their venom reservoir is significantly smaller than in uninfected wasps (Bruschini et al. 2005).

Effects of the Parasite

The manipulation of the host phenotype by the parasite, a kind of natural experiment (Beani 2006), is perhaps more relevant if we consider morphological and physiological rather than behavioural traits. Regardless of the time of year and parasite load, styloped wasps had less developed ovaries than healthy aggregated wasps, even though the latter were not in their reproductive phase. This effect is mostly due to the inhibition of the corpora allata, the juvenile hormone-secreting organs, by the parasite (Strambi & Girardie 1973; Beani 2006).

Parasitized wasps were smaller than healthy ones (Fig. 5), as reported in other studies (Turillazzi 1980; Cervo et al. 2008). The main target of infection in May and June is likely to be the first offspring of females, that is, small putative workers and not large future queens, which emerge later. This is in agreement with the paucity of infected males in aggregations (Beani 2006). On the other hand, recent evidence of a bivoltine cycle of *X. vesparum* (personal observation) implies that large late workers and sexuals

could be the target of a second infection, which is in accordance with the occurrence of a few *Xenos* females releasing triungulins from our sample. In any case, styloped wasps were smaller in prehibernating aggregations than in hibernating and posthibernating ones (Fig. 5), probably because of both the presence of a higher number of workers at the end of summer and selection for larger wasps during winter; a similar trend occurred among healthy wasps.

An additional explanation for the small size of styloped wasps may be nutrient depletion during the preimaginal stages of the host, although the cost of parasitism is bearable in terms of mortality, mass loss (Hughes & Kathirithamby 2005) and immunocompetence against pathogens (Manfredini et al. 2010b, c). A by-product of nutrient depletion caused by endoparasitism during host pupation may be the higher fluctuating asymmetry of wings found in styloped wasps. Body asymmetry is stressed by the lateral, not central, protrusion of puparium and/or cephalothorax (Fig. 1) and is consistent with the irregular and purposeless flight patterns of styloped wasps and bees as described by some authors (Linsey & McSwain 1957; Beani & Massolo 2007). Moreover, developmentally stable individuals may be less exposed to parasites than unstable ones (Thomas et al. 1998).

Alterations in physiology and morphology of styloped wasps may depend on the level of infection. Wasps infected by one or two *Xenos* females had as many fat bodies (Fig. 2e, f) as the healthy individuals (Fig. 6) and they were likely to survive until next spring. Although our ranking method is qualitative (more recent techniques are quantitative, see Toth et al. 2009), the decrease in fat bodies across months is significant and may be explained in terms of energy consumption during the winter diapause in both unparasitized and parasitized wasps. In fact, once extruded, *Xenos* females become unable to absorb nutrients from the host haemolymph and survive by means of their own adipocyte reserves (Giusti et al. 2007). Conversely, lipid storage is lower (monolayered adipocytes, Fig. 2d) if wasps are infected by many parasites or, at least, one *Xenos* male (Fig. 6). These specimens did not overwinter

(Fig. 4), similarly to workers and males: in laboratory conditions they die during October (personal observations). The short-term survival of wasps carrying *Xenos* males may be explained by several nonmutually exclusive reasons. While the cephalothorax is flattened, the extrusion of the puparium dramatically changes the shape of the wasp, perhaps promoting subsequent infections, especially when it is left empty after male eclosion. We could also hypothesize a higher energetic demand for the holometabolous development of the male (i.e. two further moults and the production of the puparium) versus the neotenic development of the female during the pupal stages of the host.

According to virulence trade-off models, the host is more valuable to the parasite if it lives a long life than if the parasite is a 'body snatcher' (sensu Lafferty & Kuris 2009). *Xenos* males could act as parasitoids: their short-term strategy is to inseminate a female in the summer, not to overwinter with the host until the next season. Conversely, *Xenos* females could act as true parasites, able to inhabit the host for months, although parasitic castrators have been classified as parasitoids owing to the 'reproductive death' of their hosts (see Kuris 1974; Kathirithamby 2009).

Styloped Wasps

The controversial morphophysiological and behavioural traits of styloped wasps prompted us to investigate to which caste they belong. Caste indeterminacy in *Polistes* (West-Eberhard 1996) implies that a gyne, a late-emerging female that will become a foundress the following season, and a worker differ dramatically not in their morphology, but in their physiology (Toth et al. 2009) and in the expression of behaviourally relevant genes (Toth et al. 2007). For example, even though gynes are generally larger than workers, females vary in size only by 10% in *Polistes metricus* (Haggard & Gamboa 1980) so body size is a reliable although not absolute predictor of rank (Pardi 1948; Reeve 1991; O'Donnell 1998). In summer, both castes have undeveloped ovaries, but while a worker is the target of social ovariectomy and destined to die after a few weeks of work (West-Eberhard 1996), a gyne is constrained from ovary development (Toth et al. 2009) only until the following spring. Moreover, gynes are characterized by the capability to withstand cold temperatures (Eickwort 1969; Strassmann et al. 1984) and by high lipid levels (see Toth et al. 2009).

With regard to caste, styloped wasps might represent a new phenotype. Their small size, their extremely low ovary development and their lipid storage, strongly affected by the number and the sex of the parasites, do not represent reliable indicators of caste (Fig. 6). To date, we assume that both workers and gynes may cluster together and overwinter if infected by one (rarely by two) *Xenos* females. Differently from workers, they leave the nest 1 or 2 weeks after their emergence, to gather, like males, on traditional sites chosen as nuptial arenas (bushes, hedges and other landmarks) or on specific flowering plants; thereafter, they cluster on buildings and then within sheltered hibernating sites like gynes. That is, styloped 'crazy' wasps follow an altered schedule, resembling in some ways the behaviour of sexuals (Beani 2006). The strepsipteran parasite exploits the gregarious behaviour of the host outside the colony and redirects nutrients from the direct and indirect fitness of the host towards its own reproduction and dispersal. The subtle selfish deformation of the host phenotype by the parasite is 'just one of the very large category of developmental manipulations by outsiders' (West-Eberhard 2003, page 458).

Both sexes of the parasite may deeply change the behaviour and the fate of the host, from eusociality to gregariousness, from the brief life span of workers (2–3 months) to the long survival of gynes (about 1 year including diapause). But the apparent

'lengthened life cycle' of the styloped host claimed by Kathirithamby (2009) may be more appropriate in the case of hornets (*Vespa*), where caste determination is clearcut and both workers and queens overwinter if parasitized (Makino & Yoshiharu 1998). Longevity of workers in the absence of nest activity is well known in *Polistes*, for example in *Polistes fuscatus*: a relevant fraction of first-brood females may disperse within a few days after eclosion, enter early diapause and become nest foundresses the next season (the alternative reproductive strategy advanced by Reeve et al. 1998). Moreover, sexuals could also be the target of a second infection, especially in the case of a bivoltine cycle. Parasitized males might join healthy males at leks (see Polak 1993b: 5% of *Polistes canadensis* males at leks were styloped; see Dunkle 1979), while putative gynes/workers harbouring the parasite may form aggregations. This bizarre parasite-parasitoid profits from the flexible caste system of the primitively eusocial *Polistes* wasp and breaks all the traditional rules of the colony by shaping a new morphological and behavioural phenotype.

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